Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management (MINA)

Philosophiae Doctor (PhD)
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The effect of biochar in combination with mineral or organic fertilizers on crop production in Nepal

Effekten av biokull i kombinasjon med mineralsk eller organisk gjødsling på produksjon av matvekster i Nepal

Naba Raj Pandit

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PhD Supervisors

Prof. Gerard Cornelissen (Main supervisor)
Faculty of Environmental Sciences and
Natural Resource Management (MINA)
Norwegian University of Life Sciences
P.O.Box 5003, N-1432 Ås, Norway
Gerard.Cornelissen@ngi.no

Department of Environmental Engineering
Norwegian Geotechnical Institute
P.O.box 3930 Ullevaal stadion
Oslo, Norway

Prof. Jan Mulder (Co-supervisor)
Faculty of Environmental Sciences and
Natural Resource Management (MINA)
Norwegian University of Life Sciences
P.O.Box 5003, N-1432 Ås, Norway
Jan.mulder@nmbu.no

Sarah Elizabeth Hale, PhD (Co-supervisor)
Department of Environmental Engineering
Norwegian Geotechnical Institute (NGI)
P.O.box 3930 Ullevaal stadion,
Oslo, Norway
sarah.hale@ngi.no

Ramji Neupane, PhD (Co-supervisor)
Nepal Agroforestry Foundation (NAF)
Koteshwor,
Kathmandu, Nepal
ramjineup@yahoo.com

Thesis Evaluation Committee

Professor Stefaan De Neve (Opponent 1)
Department of Soil Management, Ghent University
Coupure links 653, B-9000 Gent, Belgium
stefaan.deneve@ugent.be

Professor Roshan M. Bajracharya (Opponent 2)
Dept. Environment. Science and Engineering, Kathmandu University
Dhulikhel, Kavre, Nepal
roshan.bajracharya@gmail.com

Susanne Eich-Greatorex, PhD (Coordinator and Secretary)
Faculty of Environmental Sciences and
Natural Resource Management (MINA)
Norwegian University of life sciences
P.O.Box 5003, N-1432 Ås, Norway

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Summary in English

The majority of poor people in Nepal relies on agriculture for employment and livelihood sustenance. Declining soil fertility and ongoing climate change are the key challenges faced by farmers, with adverse effects on crop yield and food security. Population densities continue to increase and resources available for maintaining people's livelihood are becoming increasingly scarce. Biochar is a carbon rich material produced by heating biomass in low oxygen environment known as pyrolysis. Biochar addition in soil has been reported to mitigate climate change and increase crop production per unit of land resulting in improved livelihoods in rural tropical settings.

Biochar can be produced from different organic feedstocks and by various kiln types. Some of the previous studies have used feedstock materials such as wood, palatable grass or shrubs and other crop residues that also can be used for other purposes. Such competition for biochar feedstock may threaten the sustainability of its implementation. Therefore, organic waste not used for other purposes or non-palatable weeds should be used for biochar production. Using invasive weeds for biochar would even turn a pest into a valuable resource.

During biochar production, various greenhouse gases (GHGs) and aerosols (smoke) are emitted to the atmosphere. In developing countries, mostly traditional low cost technologies are practiced for biochar generation, contributing to higher GHGs emissions. Therefore, production technologies with low emissions (clean burn) and good quality biochar need to be developed. In this study, we used *Eupatorium adenophorum* feedstock, an invasive, ubiquitous, unpalatable shrub with local name "Banmara" (forest killer) to produce biochar. We contributed to the development of the flame curtain kiln technology to make biochar, which is easy to operate, cheap and fast, and thus feasible to small-scale farmers. To assess the effects of biochar on soil fertility, crop production and farming economy, a soil representative of Nepal's mid-hills (a silty loam moderately acidic soil from Rasuwa) was used in greenhouse and field trials.

In the first part of the thesis (**paper I and paper II**), we extensively tested this novel, clean, fast, and easy method for biochar generation, the flame curtain kiln. Seven different types of kiln to make biochar were used; four sub-types of the novel flame curtain kiln (deep metal cone, steel shielded soil pit, soil pit and small cone kiln), a brick-made traditional kiln, a traditional earth-mound kiln and a top-lit up draft kiln (TLUD). Gas and aerosols emissions such as carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4), non-methane volatile organic carbon (NMVOC), nitric oxides (NO_x) as well as quality of biochar (surface area and organic carbon content) produced from flame curtain kilns were compared to that with other traditional (non-retort) and retort kilns (paper I). Biochar

produced from these kiln types were further explored under greenhouse pot trials with maize plants to assess their agronomic effect (paper II). In addition, biochars were pretreated with hot or cold mineral nutrient enrichment (mixing with a nutrient solution before or after cooling down, respectively), or added separately at the same nutrient dosages to the soil.

Biochar produced from flame curtain kiln showed good quality biochar with high carbon contents, high cation exchange capacity (CEC), surface area (SA) and low polycyclic aromatic hydrocarbons (PAHs). The flame curtain kilns showed significantly lower emissions of CO, NO_x and total products of incomplete combustion (PIC) than non-retort (traditional) or retort kilns. No significant differences between kiln types were observed with regard to effect of biochar on maize biomass production. Thus, biochar produced from flame curtain kilns had the same agronomic effect as biochar made by the other kilns. Hot nutrient enrichment showed a significantly stronger positive effect on maize biomass than cold nutrient enriched and non-enriched biochar (with the same amounts of biochar and nutrients added separately). Hot nutrient-enriched biochar (1% w: w biochar) increased biomass by 53% and 109% compared to cold nutrient-enriched biochar and non-enriched biochar respectively.

In these experiments, biochar addition showed improved soil physicochemical properties such as moisture content (from 7 to 40 % vol.), plant available water (from 21 to 26 % vol.), pH (from 5.3 to 6.6), CEC (from 7 to 12 cmolc kg^{-1}), exchangeable K^+ (from 0.26 to 1.75 cmolc kg^{-1}) and other base cations (Ca^{2+} and Mg^{2+}), total organic carbon content (from 1.35 to 2.94 %) and plant available phosphorous (from 11 to 84 mg kg^{-1}). However, it is often difficult to pin point exactly what effect explains biochar's effect on soil fertility and crop growth, as it varies with soil type and the most important soil constraints. To determine the main mechanism responsible for the effect of biochar on crop yield in the silty loam used throughout this thesis, I focused primarily on three potential physicochemical soil limitations for maize growth i.e. water stress, nutrient stress and acid stress (**paper III**). A mechanistic study was done under controlled greenhouse conditions, using three dosages of biochar (0, 0.5% and 2% w: w) in combination with four different dosages of NPK fertilizer, water and lime. Nutrient stress was created by NPK fertilizer addition at four dosages ranging from very low amounts up to the recommended dosages. Water stress was created by watering at four amounts below those provided by normal rainfall and acid stress was alleviated to variable extents by liming (powdered $CaCO_3$) at four dosages to a previously tested range of pH values. Biochar amendment showed significant positive effects on maize biomass at all watering rates, however, its effect was less strong under water-stressed conditions (+67%) than in the presence of ample water (+311%). So, in this soil biochar did increase soil moisture, but this was nonetheless not the main reason for increased biomass growth. In contrast, biochar addition showed stronger effect under nutrient-stressed conditions (+363%) than at high, recommended nutrient application rates (+132%), indicating a strong effect of biochar on nutrient stress alleviation. This was confirmed by significant positive relationship between maize biomass and K supply rates *(R^2=0.51, P<0.001)* as well as between maize biomass and P-AL *(R^2=0.61, P<0.001).* It was concluded that soil available K and P were probably the main limitations to biomass production in this soil. Biochar addition increased soil pH, but liming and pH did not show any effect on maize biomass, so acidity stress alleviation was not the mechanism of biochar effects on soil fertility. This may be due to higher soil pH without biochar (> 4.5) than the pH where Al toxicity (acidity stress) to plant roots may be expected (pH < 4.2).

The combination of biochar with organic amendments (compost) has been suggested as a more effective and sustainable means to improve agricultural productivity and to mitigate climate change than its application together with energy-intensive inorganic fertilizers. Obtaining expensive, import based mineral fertilizer is a challenge for many tropical smallholder farmers. This work tested for the first time whether organic nutrient transformation techniques based on locally available materials (manure, greenwaste, advanced biochar) can increase the fertilizing efficiency of the resulting substrate. In **Paper IV**, we focused on three different composting methods both in the absence (compost alone) and presence of biochar (co-composted), investigating the optimal use of organic nutrients from green waste and farmyard manure: i) conventional composting (maturation without turning the piles), ii) aerobic composting (maturation under frequent pile turning) and iii) bokashi composting (fully anaerobic lacto-fermentation). A pot trial was carried out to investigate the agronomic effect of the compost only, co-composted biochar-compost mixtures and biochar-compost mixtures blended upon amendment ("post-mixed", i.e. mixed after composting) produced from these three composting methods. These organic amendments were compared to other treatments receiving the same amounts of mineral nitrogen, phosphorous and potassium (NPK; at available nutrient loadings equivalent to those in compost and co-compost). Co-composted bokashi (60 t ha^{-1}) significantly *($p<0.001$)* increased biomass production per pot by 243%, 204% and 149% compared with NPK, NPK+BC and bokashi without biochar respectively. In contrast, compost and biochar-compost mixtures (both post mixed and co-composted) produced from conventional and aerobic systems did not reveal significant effects on biomass production compared to NPK (control) and NPK+BC. Part of the explanation for the strong effect of the co-composted biochar-bokashi formulation was that much higher P-AL was observed for bokashi co-composted biochar (105 mg kg^{-1}) than for all other organic amendments and inorganic amendments with and without biochar (ranging from 32 to 55 mg kg^{-1}). Similarly, soil moisture content, CEC and exchangeable base cations (K^+, Ca^{2+}, Mg^{2+}) were observed to be highest for bokashi co-composted biochar. Bokashi fermentation uses lacto bacilli bacteria, which convert sugar into lactic acid and interact with the soil-plant environment in a complex manner to suppress plant pathogens and diseases and optimize soil nutrient availability and crop growth. Our work demonstrated that subsistence farmers in tropical countries can improve their on-farm organic nutrient management to achieve fertilizer efficiencies comparable or even better than mineral fertilizer.

In **paper V**, we investigated the effect of the same biochar on crop production in the same soil in extensive long-term field trials. To this end, we investigated six different dosages of biochar (control, 5 t ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1}, 25 t ha^{-1} and 40 t ha^{-1}) over three years in a maize-mustard cropping system. Biochar addition did not show significant effects on maize and mustard grain yield in the first year but significant positive effects ($p < 0.05$) during the second and third year crop harvest were observed. During the second year, maize grain yield significantly increased by 50%, 47% and 93% and mustard grain yield by 96%, 128% and 134% at 15 t ha^{-1}, 25 t ha^{-1} ,and 40 t ha^{-1} biochar addition, respectively. A similarly significant trend in yield of both crops was observed in the third year. The crop yield effects could be explained through significant positive linear relationships ($p<0.001$) between crop yield (for both maize and mustard) and plant available P, K^+, pH, total OC%, CEC, and soil base saturation.

On the basis of the measured crop yields for the various biochar dosages, gross margin was calculated for all the applied biochar dosages to investigate optimal biochar dosage under local farmer practices. Total cost included financial cost (farm input, labor and biochar production cost), health cost, and carbon emission cost during biochar production (including the strong greenhouse gas methane). Total income comprised sale of crops and carbon sequestration credits ranging from no carbon price (US$0 per ton CO_2), to current voluntary carbon market prices (US$6 per ton CO_2), medium social carbon cost (SCC; US$42 per ton CO_2), to a high-impact SCC (US$147 per ton CO_2). The cost-benefit analysis indicated the optimal biochar dosage to be 15t ha^{-1} for all C price scenarios with gross margin up to 42% higher with biochar use than without it.

The overall conclusion from this thesis is that flame curtain kilns are suitable for producing biochar from the ubiquitous pest shrub *Eupatorium* in a cost-effective and easy manner. Application of this biochar can overcome nutrient limitations in a representative soil from the Nepal mid-hills, mainly by improving P and K availability. Biochar also improved soil moisture retention but the watering effect is minor compared to the effect of P and K. This way biochar can improve farming economics of smallholders in this underdeveloped part of the world.

Sammendrag på norsk

Flertallet av fattige mennesker i Nepal er avhengige av landbruk som inntekts- og matkilde. Synkende jordfruktbarhet og pågående klimaendringer er bøndenes hovedutfordringer, med negative effekter på avlinger og matsikkerhet. Befolkningstettheten øker stadig og ressursene som er tilgjengelige til å opprettholde folks levestandard blir stadig mindre. Biokull er et karbonrikt materiale som produseres ved forbrenning av biomasse uten tilgjengelig oksygen, såkalt pyrolyse. Biokulltilsetning i jord har blitt dokumentert å motvirke klimaendringene, samt øke avlingene per jordareal, noe som resulterer i bedre levestandard i landlige, tropiske omgivelser.

Biokull kan produseres fra forskjellige organiske råstoffer og med ulike pyrolysemetoder. I tidligere studier har det blitt brukt råmaterialer som trær, gress eller busker, samt annet jordbruksavfall som også kan brukes til andre formål. Konkurranse om biokullråmaterialet kan true bærekraftsperspektivet i implementeringen. Derfor bør organisk avfall som ikke brukes til andre formål, eller ikke-spiselige ugresstyper, brukes til produksjon av biokull. Ved å bruke introduserte ugressarter til biokullproduksjon, vill til og med en problematisk fremmedart kunne forvandles til en verdifull ressurs.

Under biokullproduksjonen slippes ulike drivhusgasser (GHG) og aerosoler (røyk) ut til atmosfæren. I utviklingsland benyttes for det meste tradisjonelle lavkostteknologier til biokullproduksjon, noe som bidrar til høyere utslipp av drivhusgasser. Derfor må det utvikles produksjonsteknologier med lave utslipp (ren forbrenning) som gir biokull med god kvalitet. I denne studien ble det brukt *Eupatorium adenophorum* som råstoff, en introdusert, uspiselig busk med stor spredning og lokalt navn "Banmara" (skogsdreper), til å produsere biokull. Dette arbeidet var med på å utvikle "flame curtain kiln" (flammegardinovnen) til å lage biokull, som er lett å betjene, billig og rask, og dermed attraktiv for bønder som driver små-skala jordbruk. For å vurdere effekten av biokull på jordfruktbarhet, avlingsproduksjon og gårdsøkonomi, ble en representativ jord for Nepals midtre åser (en moderat sur siltig leirejord fra Rasuwa) brukt i drivhus- og feltforsøk.

I den første delen av avhandlingen (**manuskript I og II**) bleden nye, rene, raske og enkle metoden for biokullproduksjon, flammegardinovnen, testet. Syv forskjellige typer ovner ble brukt til å lage biokull; fire undertyper av den nye flammegardinovnen (dyp metallkjegle, stålskjermet jordhull, jordhull og liten kjegleformet ovn), samt en tradisjonell mursteinsovn, en tradisjonell jordovn og en liten forbrenningsovn til matlaging av typen TLUD ("Top Lit Up Draft"). Gass- og aerosolutslipp som karbondioksid (CO_2), karbonmonoksid (CO), metan (CH_4), ikke-metan-flyktig organisk karbon (NMVOC), nitrogenoksider (NO_x) og kvaliteten på biokullet (overflateareal og organisk karbon) produsert fra flammegardinovner, ble sammenlignet med utslippene og kullkvaliteten fra andre

tradisjonelle (ikke-retort) og forbedrede retortovner, som fører tilbake og forbrenner avgassene (**manuskript I**). Biokull produsert fra disse ovnstypene ble undersøkt nærmere i drivhustester med maisplanter for å vurdere agronomisk effekt (**manuskript II**). I tillegg ble de ulike biokulltypene forbehandlet med varm eller kald mineralsk gjødslingsberikelse (blanding av biokull med en næringsstoffløsning henholdsvis før eller etter avkjøling), eller tilsatt til jorda separat med de samme næringsdosene.

Biokull produsert fra flammegardinovnen hadde god kvalitetm i form av høyt karboninnhold, høy kationutvekslingskapasitet (CEC), stort overflateareal (SA) og lavt innhold av polysykliske aromatiske hydrokarboner (PAH). Flammegardinovner viste betydelig lavere utslipp av CO, NO_x og totale produkter av ufullstendig forbrenning (PIC) enn tradisjonelle ovner og retortovner. Ingen signifikante forskjeller mellom de forskjellige typene frammegardinovn ble observert med hensyn til effekten av biokull på produksjon av maisbiomasse. Således hadde biokullet produsert med flammegardinovnene samme agronomiske effekt som biokullet laget med de andre ovnene. Varm gjødslingsberikelse viste en betydelig sterkere positiv effekt på maisbiomasse enn kald gjødslingsberikelse og ikke-beriket biokull (med samme mengder biokull og næringsstoffer, tilsatt separat). Varmt, næringsberiket biokull (1 vekt-% biokull i jorda) økte biomassen med 53% og 109% sammenlignet med hhv. Kaldt, næringsberiket biokull og ikke-beriket biokull. I disse forsøkene vga biokulltilsetning forbedrede jordfysiske og -kjemiske egenskaper som vanninnhold (fra 7 til 40% vol.), plantetilgjengelig vann (fra 21 til 26% vol.), pH (fra 5,3 til 6,6), CEC (fra 7 til 12 $cmol_c$ kg^{-1}), utbyttbar K (fra 0,26 til 1,75 cmol kg^{-1}) og andre basekationer (Ca^{2+} og Mg^{2+}), totalt organisk karboninnhold (fra 1,35 til 2,94%) og plantetilgjengelig fosfor (fra 11 til 84 mg kg^{-1}). Imidlertid er det ofte vanskelig å fastslå nøyaktig hvilke av disse positive endringene i fysiske og kjemiske jordegenskaper som best forklarer effekten biokull har på jordfruktbarhet og plantevekst, da den varierer med jordtype og de viktigste faktorene som begrenser jordfruktbarhet. For å bestemme hovedmekanismen som er ansvarlig for effekten av biokull på maisavlinger i den typen siltig leirejord brukt i hele PhD-prosjektet, ble det fokusert primært på tre potensielle fysisk-kjemiske jordbegrensninger for maisvekst, dvs. vannstress, næringsstress og syrestress (**papir III**). En mekanistisk studie ble utført under kontrollerte drivhusforhold, ved bruk av tre doseringer biokull (0, 0,5 og 2 vekt-%) i kombinasjon med fire forskjellige doseringer av NPK-gjødsel, vann og kalk. Næringsstress ble skapt ved tilførsel av NPK-gjødsel i fire doser fra svært lave mengder opp til anbefalte doser. Vannstress ble skapt ved å vanne med fire ulike mengder som var lavere enn normal nedbørsmengde og syrestress ble lindret i varierende grad med kalking (pulver $CaCO_3$) ved fire doser til et tidligere testet område av pH-verdier. Biokullet viste signifikante, positive effekter på maisbiomasse ved alle vanningsgrader, men effekten var mindre sterk under vannstress (lavest vanntilførsel; +67% biomasse) enn ved rikelig vanntilførsel (+ 311% biomasse). Biokull øker altså jordfuktighet i denne jordtypen, men dette var ikke den viktigste årsaken til økt biomassevekst. I motsetning til avtagende effekt under vannstress viste biokulltilsetningen sterkere effekt under næringsstressede forhold (lav NPK; +363% biomasse) enn ved høye, anbefalte næringsstoffdoseringer (+132% biomasse), noe som indikerer at den sterke effekten av biokull på biomasse hovedsakelig ble forårsaket av lindring av næringsstress. Dette ble bekreftet av signifikante, positive forhold mellom maisbiomasse og K-opptakshastigheter ($P<0,001$), samt mellom maisbiomasse og tilgjengelig fosfor ($P <0,001$). Det ble konkludert med at jordtilgjengelig K og P vsannsynligvis var hovedbegrensningene til biomasseproduksjon i denne jorda. Biokullrtilsetningen økte også jordas pH, men kalkning og pH ga ingen effekt på maisbiomasse, så lindring av syrestress var ikke mekanismen bak biokulleffektene på jordfruktbarhet. Dette kan skyldes at jord-pH uten biokull (> 4.5) allerede var høyere enn pH der Al-toksisitet for planterøttene oppstår (pH < 4.2).

Kombinasjonen av biokull med organisk gjødsling (kompost) har blitt foreslått som et mer effektivt og bærekraftig tiltak for å forbedre landbruksproduktiviteten og enn anvendelsen av biokull beriket med energiintensivt, uorganisk gjødsel. Å skaffe dyr, importbasert mineralgjødsel er en utfordring for mange tropiske småbønder. I dette arbeidet ble det for første gang testet om gjenvinning av næringsstofferfra lokalt tilgjengelige organiske materialer (dyregjødsel, grønt avfall, avansert biokull) kan øke virkningsgraden mht. gjødsling i det resulterende substratet. I **manuskript IV** ble det fokusert på tre forskjellige komposteringsmetoder både i fravær- (kompost alene) og i tilstedeværelse av biokull ("med-kompostering"). Optimalt bruk av organiske næringsstoffer fra grønt avfall og kumøkk gjennom tre forskjellige komposteringsmetoder ble undersøkt: i) konvensjonell kompostering (modning uten å vende på komposthaugene), ii) aerob kompostering (modning under hyppig vending av haugene) og iii) bokashi kompostering (fullt anaerob lakto-fermentering). Et veksthusforsøk ble utført for å undersøke den agronomiske effekten av med-kompostert biokull vs. "etterblandet" biokull/kompost (dvs. blandet etter kompostering), med kompost fremstilt gjennom de tre ulike komposteringsmetodene. Disse behandlingene med organiske næringsstoffer ble sammenlignet med behandlinger som inneholdt samme mengder mineralsk nitrogen, fosfor og kalium (NPK, ved mengde tilgjengelige næringsstoffer tilsvarende de i kompost og med-kompost). Bokashi (60 tonn per ha) økte biomasseproduksjonen med 243%, 204% og 149% sammenlignet med henholdsvis NPK, NPK + biokull og bokashi uten biokull. Biokull-kompostblandinger (både etterblandet og med-kompostert) produsert ved konvensjonell og aerob kompostering, viste ingen signifikante effekter på biomasseproduksjon sammenlignet med NPK (kontroll) og NPK + biokull. En del av forklaringen på den sterke effekten av den med-komposterte biokull-bokashi-formuleringen var mye høyere P-AL for med-kompostert biokull/bokashi (105 mg kg^{-1}) enn for alle andre organiske og uorganiske tilsetninger med og uten biokull (mellom 32 og 55 mg kg^{-1}). På samme måte ble jordfuktighet, CEC og utbyttbare basekationer (K, Ca, Mg) observert å være høyest for med-kompostert biokull/bokashi. Bokashi-fermentasjon bruker laktobacilli-bakterier som omdanner sukker til melkesyre og interagerer med jordmiljøet på en kompleks måte for å undertrykke plantepatogener og sykdommer. Vårt arbeid viste at ved å forbedre deres organiske næringsstofforvaltning på gårdene kan småskala bønder i tropiske land oppnå gjødselvirkninger som er sammenlignbare eller enda bedre enn mineralgjødsel.

I **manuskript V** ble det sett nærmere på effekten av samme biokullet som ble brukt i veksthusforsøkene på avlinger i samme siltige leirejorda i omfattende, langsiktige feltforsøk. Til dette formålet ble det undersøkt seks forskjellige doseringer biokull (kontroll, 5 t ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1}, 25 t ha^{-1} og 40 t ha^{-1}) over tre år med vekslende dyrking av mais og sennep. Biokulltilsetningen viste ingen signifikante effekter på avlingene av mais og sennep i det første året, men det ble observert signifikante, positive effekter ($p<0,05$) på avlingene i år 2 og 3. I løpet av det andre året økte maisavlingen betydelig med 50%, 47% og 93% og sennepsavlingen med 96%, 128% og 134% ved henholdsvis 15 t ha^{-1}, 25 t ha^{-1} og 40 t ha^{-1} biokull. En tilsvarende signifikant økende trend ble observert i det tredje året. Økning av avlingene kan forklares gjennom signifikante, positive lineære relasjoner ($p<0,001$) mellom avling (for både mais og sennep) og plantetilgjengelig P, K, pH, total OC%, CEC og basemetning.

Med bakgrunn i de målte avlingene for de forskjellige biokulldoseringene ble det beregnet bruttomargin for å undersøke optimal biokulldosering for lokal jordbrukspraksis. Totalkostnaden inkluderte finansiell kostnad (innkjøp, arbeidskraft og produksjonskostnad av biokull), helsekostnad, samt karbonutslippskostnad for biokullproduksjonen (inkludert den sterke drivhusgassen metan). Samlet inntekt utgjorde salg av avlinger og karbonsertifikater, som varierte fra ingen karbonpris (US$ 0 per tonn CO_2), til dagens frivillige karbonkvotepriser (US$ 6 per tonn CO_2), til medium sosialkostnad av karbon ("social cost of carbon", SCC; US$ 42 per tonn CO_2), til en høy SCC av 147 dollar per tonn CO_2. Kost-nytte-analysen indikerte at den optimale biokulldoseringen var 15 t ha^{-1} for alle karbonprisscenarier, med bruttomargin opp til 42% høyere med biokull enn uten biokull.

Den overgripende konklusjonen fra arbeidet er at flammegardinovner er godt egnet til å produsere biokull fra den introduserte arten *Eupatorium* på en kostnadseffektiv og enkel måte. Anvendelse av dette biokullet kan øke jordfruktbarhet i en representativ jord fra de midtre åsene i Nepal, hovedsakelig ved å forbedre tilgjengelighet av P og K. Biokull forbedret også jordfuktighet, men effekten av vannretensjon var mindre enn retensjon av P og K. På denne måten kan biokull forbedre jordbruksøkonomien til småbønder i denne relativt fattige delen av verden.

List of papers

I. Emissions and char quality of flame-curtain "kon-tiki" kilns for farmer-scale charcoal/biochar production

Gerard Cornelissen, Hans Peter Schmidt, Naba Raj Pandit, Paul Taylor, Bishnu Hari Pandit, Magnus Sparrevik

PLoS ONE, May 2016, doi: 10.1371/journal.pone.0154617

II. Biochar from "Kon Tiki" flame curtain and other Kilns: Effects of Nutrient Enrichment and Kiln Type on Crop Yield and Soil Chemistry

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Hans Peter Schmidt, Gerard Cornelissen

PLoS ONE, April 2017, doi: 10.1371/journal.pone.0176378

III. Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Hans Peter Schmidt, Gerard Cornelissen

Science of the Total Environment (STOTEN), January 2018, doi:10.1016/j.scitotenv.2018.01.022

IV. Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth

Naba Raj Pandit, Hans Peter Schmidt, Jan Mulder, Sarah Elizabeth Hale, Olivier Husson, Gerard Cornelissen

Under review in European Journal of Agronomy

V. Multi-year double cropping biochar field trials in Nepal: finding the optimal dosage through agronomic trials and cost-benefit analysis

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Andrew R Zimmerman, Bishnu Hari Pandit, Gerard Cornelissen

Under review in Science of the Total Environment

1. Introduction

In recent years, soil degradation and nutrient depletion are key challenges faced by farmers in different region of the world including Nepal. This has resulted in reduced crop production per unit of land affecting livelihoods and food security. Climate change is another threat affecting crop production, especially where farmer practices depend on rain-fed agriculture to sustain their livelihoods (Maraseni, 2012). According to FAO 2009, global food production needs to be increased by 70% to feed the additional 2.3 billion people by 2050. To address the two major global issues i.e. climate change adaptation and halting soil degradation and nutrient depletion, both important for ensuring sustainable food security, efficient soil management strategies including conservation of soil organic matter (SOM) have to be developed (Chan et al., 2008). In recent years, biochar has been suggested as a soil enhancer in low productive soils, where it has been reported as a sustainable technology for the restoration of SOM (Lehmann et al., 2006). SOM restoration through biochar amendment not only improves soil fertility (increased soil moisture and nutrient retention, pH, CEC), but also acts as a potential soil carbon sink (Chabbi et al., 2017), due to its recalcitrant nature (not easily decomposed by microbes) and slow chemical transformations (Kuzyakov et al., 2009). This will result in a long-term global carbon sink, which will benefit the environment and may contribute to the recent global initiative targeting 0.4% of soil carbon sequestered per year (Chabbi et al., 2017). Increasing soil C of all global agricultural land by 0.4% annually, this would offset all fossil C emissions (Chabbi et al., 2017). In addition, biochar reduces the emissions of greenhouse gases (GHGs), such as nitrous oxide (N_2O) (Obia et al., 2015) and decreases leaching of inorganic fertilizers, which require large amounts of energy to synthesize (Shrestha and Pandit, 2017). Thus, improved soil fertility and SOM pools upon biochar amendment may create a potential platform for sustainable agricultural diversification or intensification and resilience to climate hazards, i.e. climate change adaptation. This has shown positive impact on sustainable livelihood economy through improved food security and reduction of poverty, conflict and migrations (Chabbi et al., 2017; Wischnath and Buhaug, 2014).

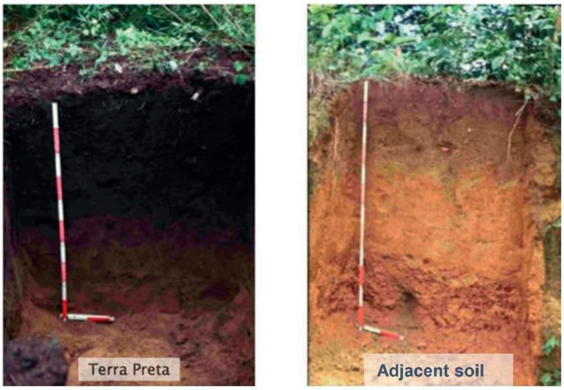

Fig 1. Soil profile with biochar amended "Terra Preta" soils (*left image*) and non-biochar soils (*right image*), Source; (Glaser et al., 2001)

Biochar is a carbon rich material produced by the pyrolysis of biomass such as wood, leaves, stems or manure i.e. heating the biomass in the partial or complete absence of oxygen (Lehmann, 2007a). Application of biochar in soil is not a new concept (Lehmann et al., 2006), as it was practiced a long time ago by Amerindian populations (Erickson, 2003). Presence of biochar or charcoal and other organic household waste in Amazon dark earth soils (man-made soils) commonly known as " Terra Preta de Indo " since prehistoric times (around 2500 years ago) sustained fertility along with higher amount of organic carbon (Glaser et al., 2001; Lehmann et al., 2007) compared with adjacent soils in the absence of biochar (Fig .1). As a result, the most infertile Amazon soils were transferred into relatively productive soils. These biochar-amended soils are still more fertile and contains more SOC than adjacent non-amended soils, which illustrates the long-term carbon stability and the long-term soil fertility improvement of biochar.

Biochar has multiple benefits with respect to environmental management; soil improvement and land use, climate change abatement, as well as pollutant immobilization, energy production and waste management (Fig .2, Lehmann et al. 2009). Biochar amendment improves soil physicochemical (Cornelissen et al., 2013a; Martinsen et al., 2014; Obia et al., 2016) and biological properties (Atkinson et al., 2010) leading to sustained soil fertility and nutrient use efficiency in highly weathered nutrient poor soils (Lehmann and Joseph, 2015). With respect to climate change mitigation, biochar is highly recalcitrant in nature, thus, acting as a carbon sequestration technique (negative emissions technology) that can store carbon in soil for several hundreds of years (Gurwick et al., 2013; Lehmann et al., 2006). This will lead to reduced CO_2 emission from the soil, combatting with the increase of CO_2 in the atmosphere, which is closely related to rising global temperature

(Solomon 2007; IPCC 2007). In view of the target of maximum 2 ^{0}C global temperature rise, biochar amendment could, similar to bioenergy carbon capture and storage (BECCS), serve as a potential negative emissions technology (NETs). In addition, biochar amendment also reduces other potential green house gas (GHG) emission from soil such as nitrous oxide (Clough et al., 2013; Obia et al., 2015) and methane (Liu et al., 2011).

Biochar addition also may reduce the bioavailability, emission and leaching of harmful chemical pollutants (for e.g., pesticides) in contaminated soil through strong sorption in nano-pores in high surface area biochar, thus, maintaining healthy ecosystem (Graber et al., 2012). Organic waste and by-products (such as manure) from animals (Uzoma et al., 2011) and crops (Chan et al., 2008) could be efficiently managed through valuable biochar production. Organic waste management can reduce methane emission from landfills and rice husk at rice polishing mills, recover energy from waste and reduce energy for long distant waste transportation (Woolf et al., 2010). Furthermore, during biochar production, energy is generated, which can be effectively used as source of bioenergy reducing the overall emissions from fossil fuels (carbon neutral energy) (Lehmann, 2007a).

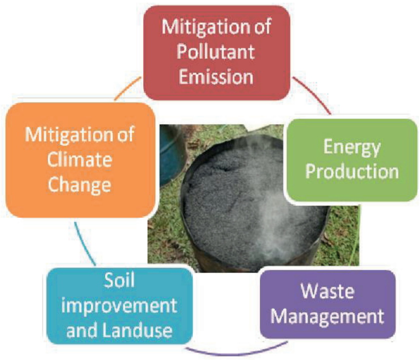

Fig.2. Multiple benefits of biochar (source, Lehmann et al. 2009)

1.1. Biochar properties

With respect to physical properties, biochar has high specific surface area (SSA) with high adsorption capacity and affinity for various compounds (mainly organic compounds (Hale et al., 2016) but also heavy metals, especially Pb, Cu and As (Ahmad et al., 2014)) high porosity of various sizes and low bulk density (Abdullah and Wu, 2009; Lee et al., 2013). Porosity and SSA of biochar can vary significantly with biomass type (Lee et al., 2013) and pyrolysis temperature (Budai et al., 2014). The biochar produced from stem wood and bagasse has shown higher porosity and SSA compared with that made from paddy straw (Lee et al., 2013). Biochar production under high pyrolysis temperature (>500^{0}C) has higher SSA compared to the biochar generated at lower pyrolysis temperature (Manyà, 2012). However, Budai et al. (2014) reported maximum SSA at the pyrolysis temperature of 600 - 700^{0}C, after which porosity and SSA begin to decline with a further rise in temperature, due to disintegration of pore structures (Hao et al., 2014).

With regard to chemical properties, biochar is mostly alkaline in nature (high pH), usually ranging from pH 6 (near neutral) to pH 10 (Jeffery et al., 2011). Biochar has shown high cation exchange capacity (CEC) (Cornelissen et al., 2013a; Martinsen et al., 2014) and low anion exchange capacity (AEC) (Mukherjee et al., 2011), due to its negative surface charges (Manyà, 2012). In addition, biochar has high organic carbon content (OC; 40-90%) and the carbon yield mainly depends on pyrolysis temperature. According to European Biochar Certificate (EBC, 2012), biochar should have organic C contents > 50%. In many cases, both slow pyrolysis and high pyrolysis temperature has shown total OC% more than 50% (Manyà, 2012). Biochar produced at low pyrolysis temperatures (around 250 ^{0}C) has less aromaticity (less condensed C rings) and high oxygen content, and is relatively labile in nature (Fig.3). It also has relatively low porosity. On the other hand, biochar generated at high pyrolysis temperature (above 500 ^{0}C) has high aromaticity (highly condensed C rings) and low oxygen content and is highly recalcitrant in nature (Fig.3), with a highly porous nature (Bostick et al., 2016).

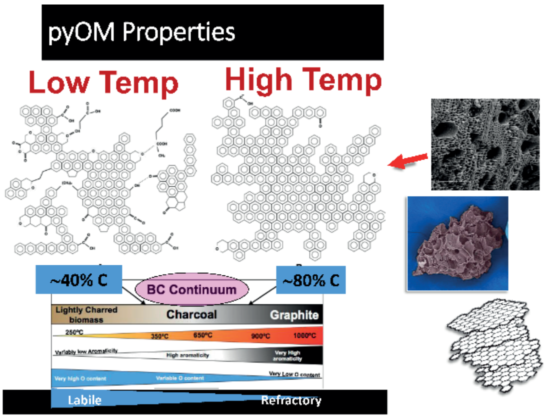

Fig.3. Biochar characteristics produced from low and high pyrolysis temperature. Source; (Bostick et al., 2016).

1.2. Biochar Production technology

Biochar can be produced from various feedstocks with different kiln types (Fig.2) and pyrolysis technologies (slow and fast pyrolysis). During biochar production, various GHGs and aerosols are often emitted. Biochar production in industrial devices produces high quality biochar with low gas emissions (EBC, 2012), but incurs high cost to operate (USD 600 to 900 per ton biochar) (Shackley, 2015), and may thus not be feasible in many rural settings in developing countries, including those in Nepal. In such a situation, the main challenges have been to introduce low cost technology that is affordable to the farmers, simple enough for them to operate, along with low emission of gases and particles during the production process (Sparrevik et al., 2015). Some of the feasible biochar production technologies could be traditional brick kiln or earth mound kiln, improved retort kilns (Adam, 2009; Sparrevik et al., 2015), top-lit up-draft (TLUD) pyrolysis units (McLaughlin, 2010) and flame curtain Kon-Tiki (Schmidt et al., 2015). These technologies will be discussed in detail below.

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Traditional brick kiln or non-retort kilns (Fig.4a) can produce biochar from different types of biomass feedstock. Pyrolysis process is slow, at moderate temperatures (300^0C - 500^0C), and biochar is produced with relatively low yield (10-20%) (Pennise et al., 2001; Sparrevik et al., 2015), however, this yield is still higher compared to that obtained at higher pyrolysis temperature with traditional methods (Manyà, 2012). Traditional kiln are cheap and easy to operate. However, toxic pyrolysis gases such as methane (CH_4), carbon monoxide (CO) and aerosols (both PM 2.5 and PM 10) are released untreated, and this leads to greenhouse gas emissions, pollutant emissions and loss of energy (Pennise et al., 2001).

Improved retorts kiln (Fig.4 b&c) introduced the partial afterburning of pyrolysis gases (Adam, 2009). Different types of organic waste feedstock (wood, rice husk, weeds, maize cobs) can be mixed and operated in the system (Sparrevik et al., 2015). Improved retort kilns have features to recirculate the produced syngases into the combustion chamber sustaining the process with less heat (pyrolysis) (Bailis, 2009), resulting in up to 75% less toxic and greenhouse gas emissions (Adam, 2009; Sparrevik et al., 2015) as well as higher conversion efficiency (up to 40 %) compared to traditional brick kiln, due to less losses of energy-rich molecules. However, improved retort has some limitations as it requires more cost, imposes technical challenges (complicated construction and operation difficulties) with slow process (2 days) and most importantly, requires large amounts of valuable startup wood in the firebox to initiate the process and warm up the kiln until the exothermic pyrolysis process commences (Adam, 2009).

Top-lit up-draft (TLUD) pyrolysis units (Fig.4d) commonly known as household-scale cooking stoves as this system can generate biochar while using the energy produced for cooking (Kumar et al., 2013).

TLUD can use wide range of organic waste feedstock that burns cleanly with reduced emissions of CO, CH_4 and aerosols by 75% compared to traditional kiln, as the syngases are combusted largely in the flame front during the process (Bailis et al., 2009). In most cases, TLUD is operated indoors reducing negative health impact to the surroundings (Smith and Mehta, 2003). However, TLUDs are relatively small producing little biochar (around 300 g per run), and may thus be feasible only for small scale horticultural systems such as kitchen gardens and intensive vegetable growing (Torres-Rojas et al., 2011).

Flame curtain pyrolysis open pit kiln "Kon-Tiki" (Fig.2 e&f) was recently developed and designed in Switzerland by Schmidt & Taylor (2014) and has many advantages over traditional kilns, improved retort kilns and TLUDs. Similar to TLUDs, it follows the principle of pyrolyzing biomass layer after layer in an open, conically built metal kiln (pyrolysis temperature around 600-700^{0}C) and is relatively cheap, fast and easy to operate. In contrast to medium-sized retort kilns, no startup wood is needed for flame curtain kilns. The flame curtain kiln allows biochar production in relatively large quantities (700 to 850 L volume biochar) within 4 - 5 hours' time (Schmidt and Taylor, 2014). The cost per kiln varies with design, construction material and country but is within a range of US$30 (soil pit shield) to US$ 500 - 1000. However, at farmers scale, flame curtain soil pit kiln (Fig.2f) would be feasible which is free of cost. Flame curtain pyrolysis kiln (all sub-types) produce good quality biochar (from Eupatorium feedstock) qualifying the premium quality of European Biochar Certificate (EBC) (Schmidt et al., 2015). One of the topics of the present thesis work was the extensive evaluation of this novel flame curtain kiln, both with regard to sustainability (gas emissions) and biochar quality.

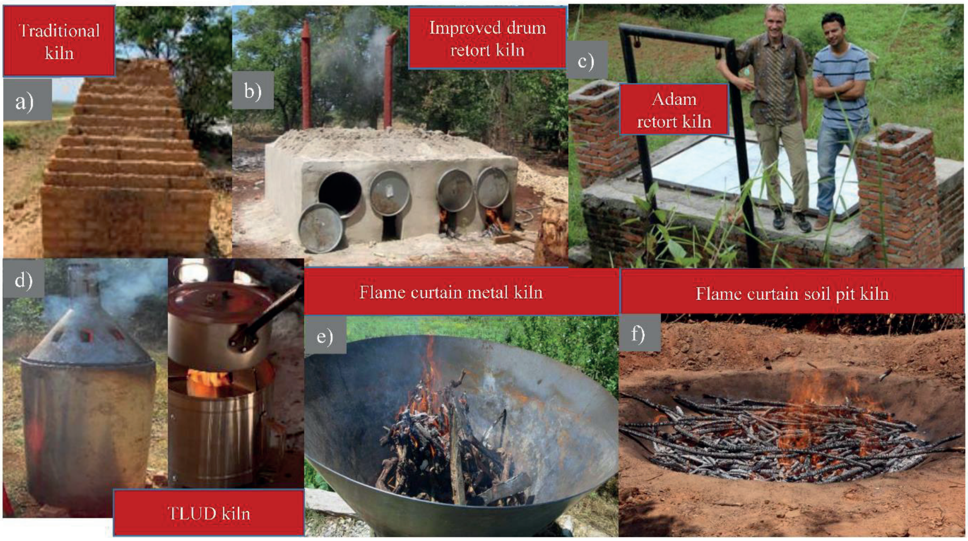


Fig.4. Biochar production technology; non-retort (*Fig a*) and retort kiln (*Fig b*) (Sparrevik et al., 2015); adam retort kiln (*Fig c*) (Adam, 2009); TLUD kiln (*Fig d*) and flame curtain kiln (metal kiln (*Fig e)* and soil pit kiln *(Fig f)*) (Schmidt and Taylor, 2014).

1.3. Effect of biochar on carbon sequestration

As mentioned above, biochar can reduce GHG emissions in three ways: i) direct C storage; ii) negative priming, i.e., the stabilization of non-biochar soil organic matter, and iii) reduced N_2O emissions. These three principles will be discussed in this section.

1.3.1. Direct carbon sequestration

The key challenge of climate change is the rising fossil fuel emissions and the fast turnover of terrestrial organic carbon, which release carbon dioxide to atmosphere thereby increasing atmospheric CO_2. In land ecosystems, biochar addition has been considered as a "negative emissions technology" (NET) (Fig.5), which sequesters carbon in soils for several hundreds to thousands of years due to its recalcitrant nature, which resist decomposition for longer periods unlike other soil organic matter that will be decomposed within months to decades (Lehmann et al., 2006). Among 74 studies explored for biochar stability (fate of biochar in soil) by Gurwick et al. (2013), mean residence time (MRT) of biochars estimated under in-situ field conditions showed an enormous span of 8 to 4000 years. However, the biochars on the low end of the stability range were mostly made at low temperatures (below 250 ^{0}C) in hydrothermal conversion processes.

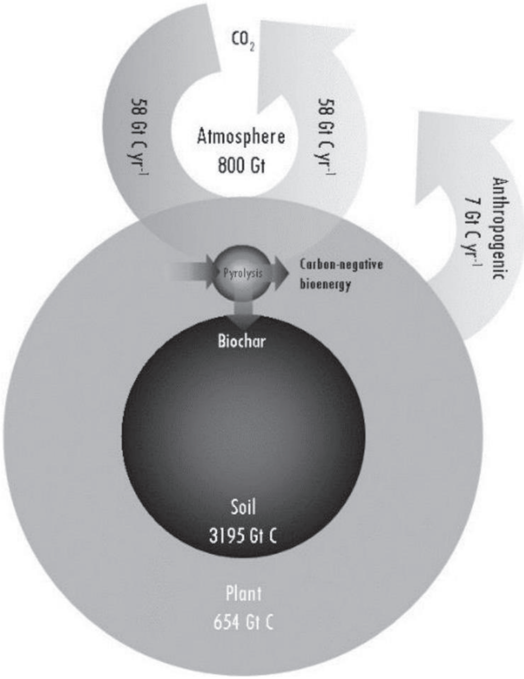


Fig.5. Global carbon cycle in terrestrial ecosystem; biochar as a negative emissions technology (NET). Source; (Lehmann et al., 2009).

The pivotal question is whether biochar can provide a significant wedge in climate change abatement on a global scale. Roughly, conversion into biochar of 12% of the global net primary production (NPP) of 58 Gton C per year and burying them into soil (long term carbon sink) would offset the increased annual atmospheric CO_2 of around 7 Gton C per year (Matovic, 2011). The global production of agricultural waste is around 9 Gton C per year (Lehmann et al., 2009). Converting 20% of this biomass to biochar, storing 50% of the biomass C, would thus offset approximately 10% of global fossil C emissions. Thus, if globally applied on a large scale, biochar could provide a significant wedge in climate change mitigation.

Kuzyakov et al. (2009) produced biochar from ^{14}C labeled plant residues (perennial ryegrass; *Lolium perene*), incubated it in soil and loess for 3.2 years and observed the rapid degradation (estimated based on $^{14}CO_2$ efflux) of 2-3% biochar C, after which degradation slowed down to mean residence times (half-lives) of 2000 y, even when glucose was added to stimulate microbial decomposition activity (Fig.6). Biochar stability mainly depends upon feedstock and pyrolysis conditions from which biochar was produced (Hamer et al., 2004; Nguyen et al., 2010). Biochar produced through corn stover and rye has shown faster rate of decomposition than that from wood (Hamer et al., 2004). In addition, soil type and environmental conditions also influence the stability of biochar (Gurwick et al., 2013; Manyà, 2012). Several short term incubation studies has shown the mineralization of biochar, both through photochemical and microbiological process (Cheng et al., 2006; Hamer et al., 2004). Thus, the stability of biochar in soil is a key factor determining the potential role of biochar for long term CO_2 sequestration (Manyà, 2012).

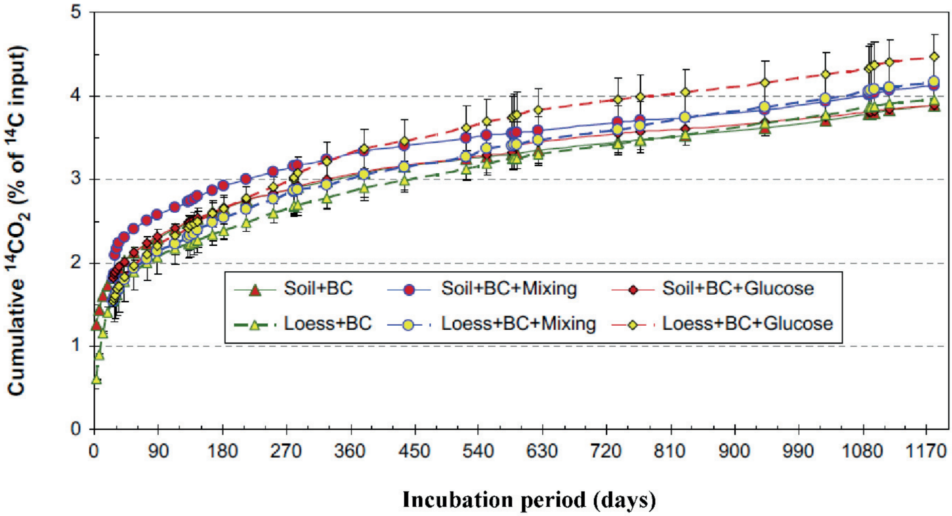


Fig.6. Black carbon (BC) mineralization (± SE) in soil and loess as affected by 4 glucose additions or intensive mixings on cumulative $^{14}CO_2$ efflux (source;(Kuzyakov et al., 2009))

1.3.2. Priming effects: effect of biochar on SOM contents

Though biochar itself is recalcitrant in nature, biochar may influence the stability of native soil organic matter (SOM) when applied in soil (Lehmann et al., 2009; Luo et al., 2011). This so-called "priming effect" of biochar (C mineralization) can be positive or negative in soil and the magnitude of C decomposition may vary with biochar type (feedstock and pyrolysis conditions), soil type (affect microbial population) and the incubation stage, ranging from -52% to 89% in one year period (Zimmerman et al., 2011). Positive priming effect of biochar (higher C mineralization and thus loss of SOM) has been observed for biochar produced from feedstock such as grasses (labile materials) at low pyrolysis temperature and during early incubation period (Hamer et al., 2004; Luo et al., 2011; Zimmerman et al., 2011). Zimmerman et al. (2011) reported positive priming effect when biochar was produced from grasses at low pyrolysis temperature of 250 and 400 ^{0}C in the early incubation stage of 90 d. In the same study, negative priming effect of biochar in soil (less C mineralization) was found when biochar was produced from hardwoods at high pyrolysis temperature (525 to 650 ^{0}C) and during later incubation period of 250-500d. Another study from Luo et al. (2011) has also shown the positive priming effect of biochar during early incubation period and when produced at high pyrolysis temperature. Biochar addition has been found to have positive priming effect in fallow soil (without vegetation) but negative priming effect in cultivated soil where priming effect was positive during early days (0-62 d) and negative during later days (62-388 d) (Weng et al., 2015). Priming effect of biochar could be positive in early stage due to the availability of reduced SOC and more labile C content (Zimmerman et al., 2011), which would enhance the microbial competition resulting in high C mineralization and release of soluble organic and inorganic in the system (Fig.7). However, in the course of time, biochar with its highly porous structure may sequester other soil organic matter and other minerals in the pores protecting it from further microbial and physio-chemical degradation (Fig.7) and thus, resulting in negative priming effect over time with aged biochar (Zimmerman et al., 2011). Negative priming effect of aged biochar would restore the carbon in soil for long periods (Table.1), highlighting its positive role in long-term soil carbon sink. This is sketched in Fig. 8: after adding a dose of biochar, a small portion of the biochar is degraded, but gradually SOM is built up ("New C"). Multiple doses of biochar will aid in the long-term buildup of SOM. A long-term field experiment by Weng et al. (2017) illustrated this well (Table 1): 8.6 y after an initial biochar amendment, a second biochar application led to relatively quick buildup of natural SOM- one year after the second amendment, SOC in the biochar plot had increased from 5000 to 5500 g C m^{-2}, while SOC contents in the control soil remained unchanged (Weng et al., 2017; table 1)

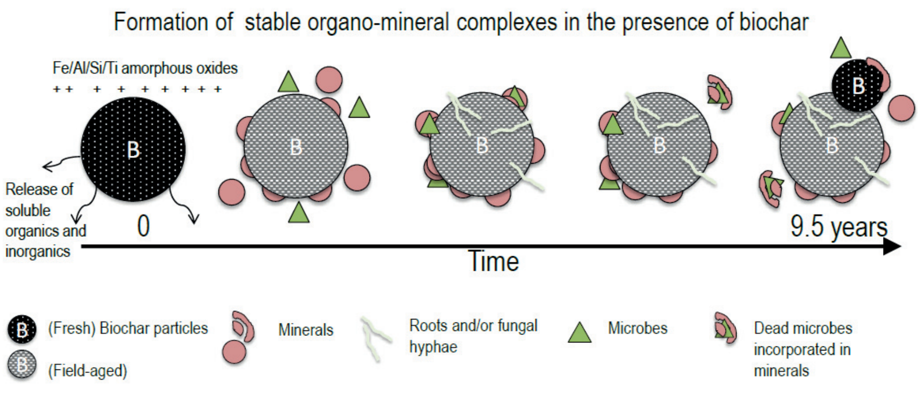


Fig 7. Formation of stable organo-mineral complexes in the presence of biochar over time (source; (Weng et al., 2017))

Table 1. Total soil carbon showing priming effect of biochar over time; Source (Weng et al., 2017)

	Total soil C (g C m^{-2})			
	8.6 yrs	8.9 yrs	9.2 yrs	9.5 yrs
Control	3.518 ± 23	3503 ± 32	3533 ± 38	3615 ± 51
Biochar	5011 ± 113	5168 ± 122	5265 ± 83	5524 ± 98

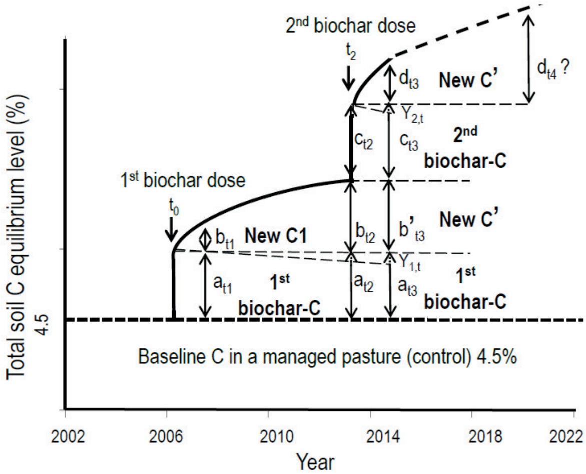


Fig 8. Biochar for long-term carbon sink (negative priming effect of biochar) increasing SOM (New C) over time; Source; (Weng et al., 2017).

1.3.3. Effect of biochar on N_2O emissions

Nitrification, denitrification and dissimilatory nitrate reduction are three major microbial processes that release reactive nitrogen such as nitric oxides (NO) and nitrous oxide (N_2O) to the atmosphere (Azam et al., 2002), which has negative impact on terrestrial ecosystem and ozone layer (Ravishankara et al., 2009; Vitousek et al., 1997). Especially N_2O is a strong GHG, with 310 times stronger heating potential than CO_2. It is the 3rd most important GHG, responsible for 10-15% of global warming, and mainly emitted from (over-fertilized) agriculture (Zhu et al., 2013). Biochar amendment in soil has shown reduced N_2O emissions (Singh et al., 2010; Zhang et al., 2010). However, the mechanism of reduced N_2O emissions is still not fully understood and a few studies even reported increasing N_2O emissions (Cayuela et al., 2014). But on the whole, Cayuela et al. (2014), in a meta-analysis, reported the drastic reduction of average N_2O emissions by 54% upon biochar amendment. Four possible mechanisms for reduced N_2O emissions upon biochar amendment have been suggested (Cayuela et al., 2014); 1) sorption of N_2O in biochar pores (Cornelissen et al., 2013b), 2) enhanced N_2O reductase activity at biochar-induced higher pH, 3) increased electron shuttling, catalysing N_2O reduction and 4) increased N immobilization and lower nitrate availability due to higher C/N ratio.

Extensive discussion of the individual mechanisms is outside the scope of this thesis, but the most important mechanism, the pH-induced increase in N_2O reduction, will be briefly described. N_2O emission has been found to be strongly dependent on soil pH conditions (Obia et al., 2015). Low pH inhibits the assembly of N_2O reductase enzyme (enzyme reducing N_2O to atmospheric N_2) (Bakken et al., 2012). Thus, a pH increase as a result of the alkaline effect of biochar may alleviate this inhibition of N_2O reductase enzyme (Obia et al., 2015). Obia et al. (2015) reported reduced net emissions of both NO and N_2O and increased N2 production upon rice husk and cacao shell biochar amendment and found a strong relationship between biochar-induced pH change and suppression of N_2O emissions (Fig.9).

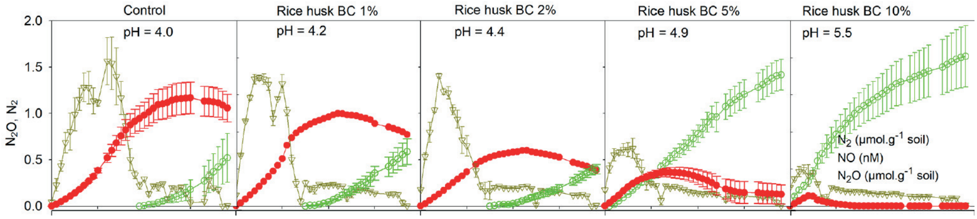


Fig 9. Emission of N_2O (denitrification kinetics) in anoxic incubation for rice husk biochar applied at different rates and control soils from Lampung, Indonesia; source: (Obia et al., 2015). The red symbols depict N_2O emissions. Green symbols are N_2 and brown symbols are NO.

1.4. Effect of biochar on soil physical properties

Physical properties of soil such as bulk density, porosity, surface area, water holding capacity (WHC), penetration resistance, water repellency and aggregate stability have been found to be improved upon biochar addition in low fertile tropical soils (Obia et al., 2017, 2016). Effect of biochar on soil physical properties depends on several factors such as feedstock type, pyrolysis conditions, biochar dosages, soil type, and environmental conditions (Mukherjee and Lal, 2013). Biochar addition has shown effects on soil physics that were more pronounced in sandy (coarse textured), acidic and tropical soils compared to clay (fine texture), neutral and temperate soils respectively. Bulk density decreased significantly by 1.28 to 1.22 g cm^{-3} upon biochar addition (1% w:w) in sandy loam in Mkushi soil, Zambia (Obia et al., 2016). In the same study, pore size distribution of soils increased (radius > 1µm) upon 2.5% biochar addition under maize crop plantation.

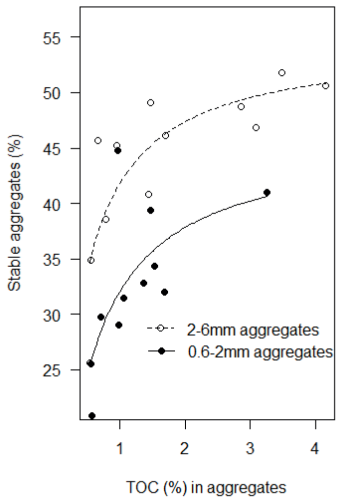


Fig 10. Stable aggregates vs biochar carbon (%) in aggregates in Mkushi soil, Zambia. Source; (Obia et al., 2016)

Several studies, thus far, has reported increased water holding capacity (WHC) of low fertile tropical soils, which often are characterized by a small WHC and plant available water (PAW) contents (Bruun et al., 2014; Dugan et al., 2010; Martinsen et al., 2014). Karhu et al. (2011) reported increased WHC by 11% upon biochar amendment (9 t ha^{-1}) in a silty loam agricultural soil. Likewise, PAW increased from 18.2% to 22.7% in a sandy loam soil in Zambia upon 10% (vol.) biochar addition (Martinsen et al., 2014). Similar trend has been observed upon 4% biochar addition where PAW increased by 3% in similar soil from Zambia (Obia et al., 2016). Increased WHC and PAW can possibly be explained by improved pore structure (both microporosity and mesoporosity) and soil aggregation upon biochar addition (Herath et al., 2013; Obia et al., 2016). Biochar amendment significantly increased soil aggregate stability by 17-20% and porosity by 2% under field trials (soybeans plantation), located in Zambia (Obia et al., 2016). This study also reported increased stable aggregate with increasing carbon% , which levelled off at a maximum of 51.4 % (for 2-6 mm aggregates) and 41.3 % (for 0.6 to 2mm aggregates) (Fig.10). In another study, soil aggregate stability increased by more than 17% upon biochar addition (10 t ha^{-1}) compared with control in a silty loam soil from Manawatu, New Zealand

(Herath et al., 2013). However, biochar addition does not always increase plant available water due to reduced hydraulic conductivity in highly porous biochar, that can hold the water at greater water potential than produced by plants (Lal and Shukla, 2004). Decreased hydraulic conductivity of sandy soil has been reported upon cow manure biochar addition (Uzoma et al., 2011).

1.5. Effect of biochar on soil chemical properties and plant available nutrients

Biochar amendment has been found to improve soil chemical properties (pH, CEC, base saturation and exchangeable K) in low productive (low pH, CEC) weathered soils (Cornelissen et al., 2013a; Liang et al., 2006; Martinsen et al., 2014). pH and nutrient effects will be discussed in this paragraph.

1.5.1. pH effects of biochar

Low pH is commonly associated with increased Al-concentrations in soil solution, which is highly toxic to plant roots (Gruba and Mulder, 2008). Gruba and Mulder (2015) also showed that the exchangeable Al concentration in acid soils reaches maximum values at pH_{H2O} below 4.2 due to the dissolution of gibbsite (Gruba et al., 2013) while declining with pH increase. The Al concentration can be reduced drastically by addition of biochar that acts as a liming agent in many acidic soils, especially if the pH can be raised to values above 4.2 (Martinsen et al., 2015; Yamato et al., 2006). When 20 t ha^{-1} biochar was applied on highly weathered tropical soils, soil pH increased from 3.9 to 5.1, thereby reducing exchangeable Al^{3+} from 2.67 to 0.12 $cmol_c$ kg^{-1} and exchangeable H^+ from 0.26 to 0.12 $cmol_c$ kg^{-1} (Yamato et al., 2006). The level of increase in soil pH was shown to mainly depend on initial soil pH and CEC as well as acid neutralizing capacity (ANC) of the biochar (Martinsen et al., 2015). Accordingly, biochar addition (10% vol.) has shown increased base saturation (BS from 7.2 to 78.2% in Mongu and from 43.4% to 90% in Mkushi) in low fertile Zambia, soil (Martinsen et al., 2014), with low-CEC acidic soils being most amenable to biochar amendment, because of the relatively modest reserve acidity, i.e., the relatively low amount of acid in moles per unit soil mass. Among various base cations, biochar amendment has been found to add significant amount of exchangeable K^+ in low fertile soil (Martinsen et al., 2014). Exchangeable K increased from 0.21 (no biochar) to 0.39 $cmol_c$ kg^{-1}, 0.56 $cmol_c$ kg^{-1} and 1.30 $cmol_c$ kg^{-1} upon 10 t ha^{-1}, 50 t ha^{-1} and 100 t ha^{-1} biochar addition respectively in an alfisol (Chan et al., 2008).

1.5.2. Nutrient effects of biochar

Biochar has different effects on the main nutrients N, P and K. While its major effect on N is increased retention, its main improvement for P is increased availability of tightly bound P in oxide-rich tropical soils. Its main effects on available K contents are increased K retention through increased CEC, but also direct addition of significant amounts of the element, as biochar is rich in K

1.5.2.1 Nitrogen and metal retention and availability.

Biochar addition has shown increased soil nutrient retention capacity, thus, reduced leaching in a low productive soil (Laird et al., 2010). For nitrate and phosphate, biochar addition (40 t ha^{-1}) mixed with swine manure has shown reduced leaching by 11% and 69% respectively (Laird et al. 2010a). In another study, biochar showed reduced leaching of ammonium, nitrate and phosphate (35%, 34% and 21%, respectively) under ex-situ conditions (Yao et al., 2012). Despite relatively low adsorption of anions (such as nitrate) to biochar due to the low anion exchange capacity of biochar (Hale et al., 2013), many studies have shown reduced leaching of nitrate (Laird et al., 2010; Yao et al., 2012). The main mechanism being the adsorption and absorption of nitrate and other nutrients in biochar organic pore coatings (Hagemann et al., 2017; Kammann et al., 2015). Biochar addition has been shown to increase NO_3^- availability as the retained nutrients in biochar pore coatings facilitate slow release of nutrients in the soil, which is easily assimilated by the plants (Hagemann et al., 2017; Kammann et al., 2015).

1.5.2.2 Phosphorous availability

Biochar addition can have a strong influence on in-situ soil nutrient availability, emphasizing its role in soil nutrient adsorption and plant availability. PO_4^- - P is tightly bound in highly weathered tropical soils that are often rich in Fe and Al oxides (Hale et al., 2013). Under such conditions, biochar addition increases soil pH and makes PO_4^- - P more bio-available in soil solution (Asai et al., 2009; Hale et al., 2013).

1.5.2.3 Potassium addition

Biochar amendment increases K availability, most possibly due to high amount of K in biochar per se (Martinsen et al., 2014) or reduced K leaching as a function of biochar amendment (Laird et al., 2010). Biochar is rich in base cations (K^+, Ca^{2+}, Mg^{2+}) and when applied in soil, most importantly adds significant amount of K^+. A recent study by Gautam et al. (2017) reported increased K^+ availability upon biochar addition (5 t ha^{-1}) in silty loam Nepalese soil. Martinsen et al. (2014) reported increased

K availability in soil and increased K content in maize plant tissue due to K addition as a function of biochar amendment.

1.6. Effect of biochar on soil biological properties

Biochar amendment has been reported to improve soil biological/microbial properties (Atkinson et al., 2010; Lehmann et al., 2011), which can have beneficial effects on soil fertility and crop production. Biochar with its high porosity and surface area can provide refuge for beneficial microorganism such as mycorrhizae (Warnock et al., 2007) (Fig.11), which bind and transfer nutrients leading to enhanced macronutrient (N and P) availability (Atkinson et al., 2010). Biochar addition has been reported to improve microbial community composition and enzymatic activities thereby increasing microbial biomass, which can explain the potential role of biochar in soil biogeochemical cycles (Lehmann et al., 2011). Increased microbial biomass and rhizobia nodulation has been reported for wide range of soil and climatic conditions upon biochar addition (Biederman and Harpole, 2013). Similarly, Kolb et al. (2009) under short term incubation, reported an increased amount of microbial biomass with increasing biochar dosages applied at five different levels (0 to 0.1 kg biochar per kg soil) in four different soil types (Mollisol, Alfisol, Entisol and a Spodosol) that were incubated at 25^{0}C and measured at 0, 1.5 and 3 incubation months. Biochar has been found more effective when enriched with organic mineral complexes, which stimulate microbial activity resulting in an improved soil quality leading to the promotion of sustainable vegetable production (Ye et al., 2016).

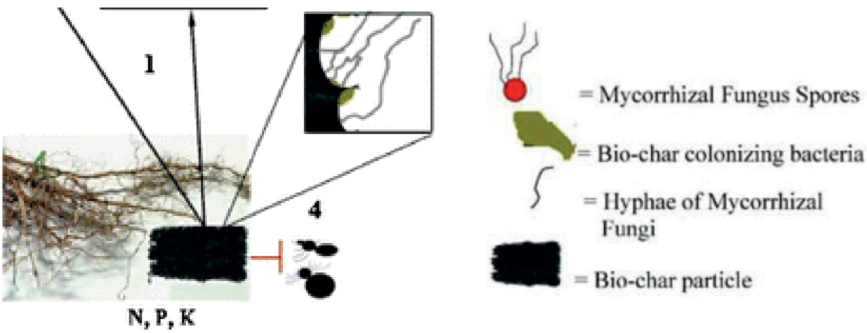


Fig 11. Effect of biochar in providing soil refuge for mycorrhizal fungi. Source:(Warnock et al., 2007)

In addition, biochar has been found to have high sorption capacity for many types of organic compounds, which reduces the availability of soil contaminants and other growth inhibitors in soil, thus, leading to favorable soil-plant-microorganism system (Hale et al., 2015; Lehmann et al., 2011). Biochar with its higher surface area adsorb and retain not only the essential nutrients but also the many organic compounds such as herbicides, pesticides or insecticides (Graber et al., 2012) and other hazardous organic compounds such as PAHs (Beesley et al., 2010), reducing the bioavailability of such toxic compounds. Furthermore, biochar amended soils have shown improved systemic resistance to some soil borne pathogens (fungal diseases); *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) in tomato and peeper (Elad et al., 2012), *Fusarium oxysporum* f. sp. *Asparagi* in asparagus (Elmer and Pignatello, 2011), *Rhizochtonia solani* in cucumber (Jaiswal et al., 2014) and bean (Jaiswal et al., 2015). Means by which biochar may influence diseases caused by soilborne plant pathogens are numerous and varied (Graber et al., 2014).

1.7. Effect of biochar on crop production

Promising effect of biochar amendment on crop growth (Fig.12) has been reported in many tropical regions; however, in many cases no or even negative effects on crop growth have been reported (Cornelissen et al., 2013a; Martinsen et al., 2014; Schmidt et al., 2015; Yamato et al., 2006). The exact mechanisms resulting in this positive yield effect is often unclear, as they vary with climate, soil type and the most important soil constraints. The elucidation of mechanisms of biochar effect on crop yield is one of the most important topics of the present research.

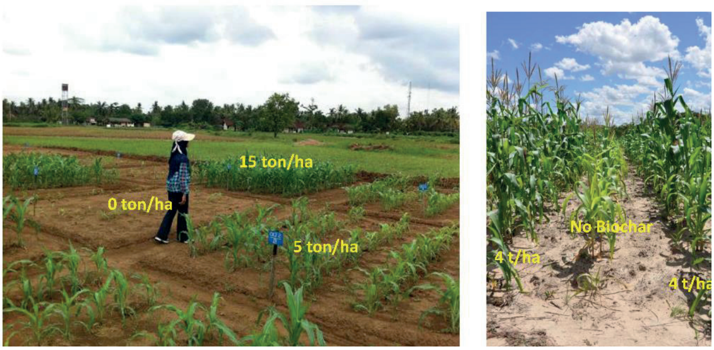


Fig 12. Illustrations of positive effects of biochar in field trials with maize crop with and without biochar addition in tropical soils; an acidic soil from Indonesia (*left image*)(Cornelissen et al., 2018, submitted) and a sandy, low-CEC soil in Zambia (*right image*); (Cornelissen et al., 2013a).

In a recent meta-analysis by Jeffery et al. (2017), biochar addition has shown average crop yield increase of 25% in tropical soils, mainly through liming (pH) and nutrient effects (N and K retention, P availability). However, their study did not include the individual assessment of soil water retention effect (PAW) on crop yield upon biochar amendment. (Martinsen et al., 2014) reported increased crop yield upon biochar addition (10% vol.) where PAW increased from 18.2% to 22.3% in Mkushi loamy soils. In his meta-analysis (Jeffery et al., 2017), he further concluded that biochar has better yield effect in tropical soil than in temperate soils, most likely because the former ones tend to be more weathered and degraded, with less optimal soil husbandry due to financial constraints. Previous meta-analysis by Biederman and Harpole (2013) has also reported more pronounced effect of biochar on crop productivity in tropical soils (+25%) than in temperate region (-5%) and stronger effects in low pH acidic soils (+40%) than pH-neutral ones (-10%), mainly through the mentioned liming effect (pH) (Fig.13). Overall, increased crop yield upon biochar addition could possibly be explained by the mechanism of liming, water retention and nutrient effect for such a low productive tropical soils (characterized as acidic, nutrient poor and coarse textured soils).

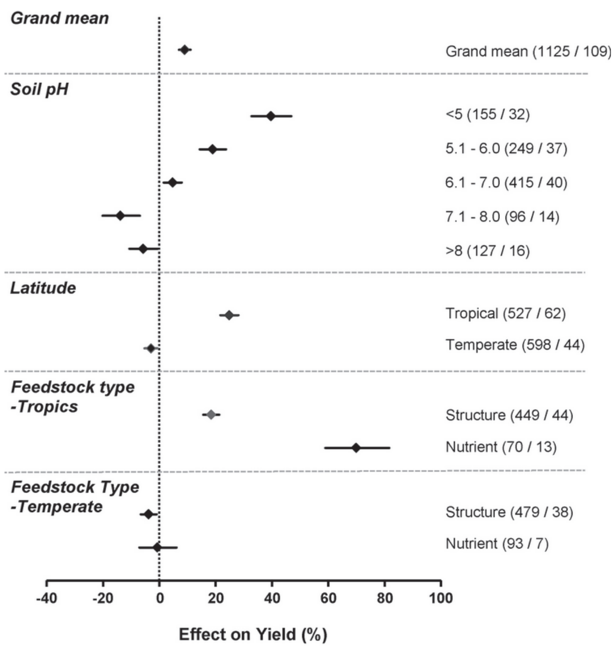


Fig 13. Biochar boosts up crop yield in low pH tropical soils. Source, (Jeffery et al., 2017)

Thus, biochar application to soil will not only contribute to the mitigation of ongoing climate change but also to socioeconomic benefit ensuring food security for the growing population worldwide (Lehmann et al., 2009). Biochar has been mainly used for two purposes by most of the tropical villagers; biochar briquettes for cooking purposes and biochar for soil amendment (Sparrevik et al., 2014). In an extensive life-cycle and cost-benefit assessment, biochar as a soil amendment showed higher economic returns and stronger environmental benefits than biochar briquettes. The better environmental effect of soil amendment was due to increased carbon sequestration in soil and higher crop production, which offset the "environmental" biochar production cost including negative environmental (gas and particulate matter emissions) and health (respiratory disease due to particulate matter emission) impact (Sparrevik et al., 2014). The better economic returns of biochar amendment to soil compared to briquetting were caused by the amount of labor involved in briquette making, as well as the higher yields returns for a similar work load during soil amendment. Thus, biochar as a soil amendment that has both carbon storage and crop yield benefits could be a sustainable and cost-effective approach for improving livelihoods of tropical rural settings worldwide (Sparrevik et al., 2014). The socio-economics of biochar soil amendment in rural Nepal will be the topic of the present study.

1.8. Biochar formulations: co-composting and nutrient-enrichment

In recent years, biochar enrichment with organic and mineral fertilizers (biochar-compost mixtures) have been gaining popularity to produce effective biochar-based slow release organic fertilizers (Schmidt et al. 2017). Biochar without enrichment, i.e. "raw" biochar, works fine for soil moisture retention or soil acidity alleviation (Martinsen et al., 2014). However, in richer soils, and in soils where nutrient retention is the most important soil fertility limitation, enrichment of biochar may be needed to attain agronomic effect (Schmidt et al. 2017). Biochar can be either mixed with composting materials during the composting process, i.e. "co-composted", or added directly to stored matured compost (post mixed biochar) (Vandecasteele et al., 2016). Addition of biochar during the composting process (co-composted BC) changes the compost properties and quality, leading to improved physicochemical properties of the harvested co-compost (Probst et al. 2013; Agegnehu et al. 2016; Vandecasteele et al. 2016). Earlier studies have shown the effectiveness of co-composted biochar in improving soil physicochemical properties (soil moisture retention, pH, CEC and other base cations) and plant available nutrients (available P, K, nitrate and ammonium) (Agegnehu et al., 2016; Ghosh et al., 2015; Schulz et al., 2013). In addition, biological properties of soil have also been improved upon biochar-compost amendments, through stimulation of microbial activity (Ye et al., 2016).

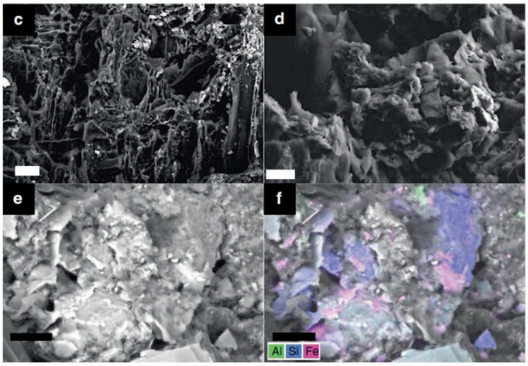


Fig 14. Identification of biochar surface modifications with scanning electron micrographs of co-composted biochar. source: (Hagemann et al., 2017).

During the composting process, an organic coating has been shown to form on the biochar, which reduces hydrophobicity of biochar and improves water and nutrient retention conditions (enriched nutrient rich compounds in inner pores and outer biochar surface, Fig.14) (Hagemann et al., 2017; Joseph et al., 2017), with beneficial effects on crop growth and root development (Agegnehu et al. 2016; Schmidt et al. 2017). Recently, Schmidt et al. (2017) conducted 21 field trials on intrinsically fertile Nepalese silty loam soils and illustrated significant agronomic effects when biochar was enriched with organic fertilizers (cow urine and manure), especially when biochar was co-composted with manure or embedded in urine for prolonged time periods so that the aforementioned organic coatings could form (Hagemann et al., 2017). In the same study, biochar enriched with organic nutrients showed an increased crop yield by 123% ± 76.7% and 103± 12.4 % compared with traditional organic fertilization and NPK-biochar fertilization respectively. Pumpkin yield increased by 306% and 85% upon amendment with the urine enriched biochar compared with only urine treatment and only biochar treatments respectively (Schmidt et al., 2015) (Fig.15). Similarly, Kammann et al. (2016) reported significant positive effect of co-composted biochar on agronomic performance compared with biochar and compost alone. In pot experiments, co-composted biochar (2% w/w) increased plant growth of *Chenopodium quinoa* by 305% in nutrient poor sandy soil compared with control (Kammann et al., 2015), in contrast to raw, non enriched biochar, that did not have any positive agronomic effects in this soil.

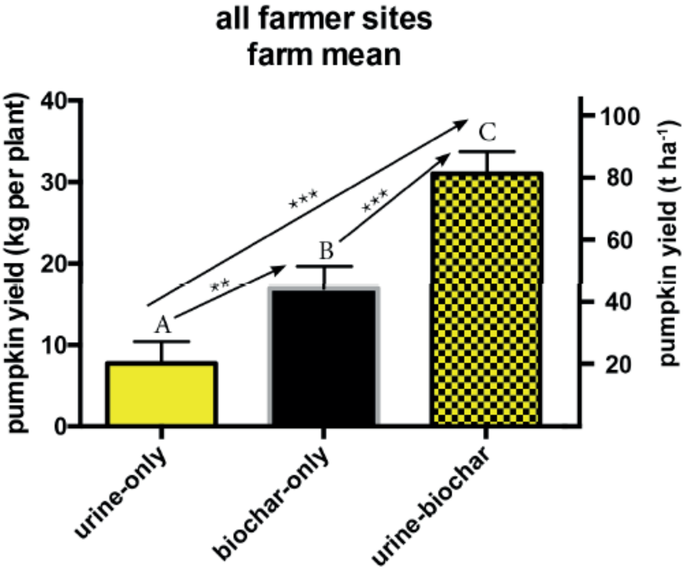


Fig 15. Effect of organic nutrient enriched biochar on pumpkin yield. Source: (Schmidt et al., 2015)

1.9. Quantification of biochar

A systematic approach is required to quantify biochar stability in soil (Gurwick et al., 2013). There are three main classes of different methods of biochar quantification (Budai et al., 2013); 1) Alpha methods, 2) Beta method and 3) Gamma methods. Alpha methods do not provide an absolute measure of stability but assess physiochemical properties related to stability, such as H/C molar ratio (Enders et al., 2012), O/C molar ratio (Spokas, 2010) and volatile matter (Enders et al., 2012; Spokas, 2010). Beta methods, on the other hand, directly quantify biochar loss over a period of time under incubation (laboratory conditions) (Zimmerman, 2010) or field studies (Kuzyakov et al., 2009). One of the limitation of Beta methods is that they are not widely accessible and time consuming (Budai et al., 2013). Gamma methods measure molecular properties associated with biochar stability.

There are various types of Gamma methods such as NMR spectroscopy (Brewer et al., 2011), pyrolysis gas chromatography mass spectrometry (PyGC/MS)-analytical pyrolysis (Fabbri et al., 2012), ring current NMR (McBeath et al., 2011) and benzene polycarboxylic acids (BPCA) (Glaser et al., 1998; Schneider et al., 2010). NMR spectroscopy measures the fraction of aromatic carbon in biochar. Py CC/MS-analytical pyrolysis uses thermal degradation to break down large molecules and the pyrolysis product measured using gas chromatography and mass spectroscopy. Ring current NMR determines the degree of aromatic condensation of biochar, which involved the sorption of ^{13}C-labeled benzene to the biochar structure. BPCA analysis gives information on both the amount of condensed, aromatic C and level of C condensation in soil. BPCA are molecules formed during nitric acid oxidation of biochar. BPCAs with varying degrees of carboxylation are identified (B3CA to B6CA with 3 to 6 –COOH substituents, respectively, indicating various levels of condensation in the original biochar matrix). Biochar forming B5CA and B6CA exhibits a higher degree of condensed aromatic rings than biochar leading to relatively more B3CA and B4CA. Thus, the ratio of B6CA-C/total BPCA-C is positively correlated with degree of condensed aromatic carbon in biochar; the larger the ratio the greater the aromaticity. Total BPCA can be used for the quantification of biochar in the environment (mixed either with soil or in mixture with other organic materials) (Budai et al., 2013).

1.10. Rationale and hypotheses of the study

The present study focuses on the utilization of the invasive forest weed "*Eupatorium adenophorum*" as a sustainable feedstock to produce biochar and to investigate the effect of produced biochar on soil fertility and crop production in a silty loam Nepalese soil. *Eupatorium adenophorum* with the local name "Banmara" (forest killer) is an invading species causing rapid destruction of forest and biodiversity, which has shown negative impact on livelihood sustainability, food security, environment and ecosystem management (Kunwar, 2003). This invasive forest shrub could be sustainably utilized to produce a valuable biochar thereby relieving an environmental problem and turning a pest into a resource.

There are several traditional low cost technologies (earth mound kiln, brick kiln), which have been used to produce biochar in many developing countries. However, these methods are not efficient and contribute to air pollution by release of syngases to the atmosphere, including methane (CH_4), carbon monoxide (CO), nitrogen oxide (NO_x), aerosols ("smoke" or particulate matter (PM); $PM_{2.5}$ and PM_{10}) as well as non-methane volatile organic matter (NMVOC), in addition to hydrogen (Pennise et al., 2001). Thus, to circumvent such challenges, the flame curtain, open pit "Kon-Tiki" kiln has been developed, which is a relatively low cost technology with clean burn of organic waste (Schmidt and Taylor, 2014).

It was hypothesized that biochar produced from Eupatorium feedstock using flame curtain kilns would produce biochar of good agronomic quality and with low amounts of deleterious compounds, and with low gas/syngases and particle emissions. This hypothesis was tested in Paper I entitled "Emissions and char quality of flame-curtain "kon-tiki" kilns for farmer-scale charcoal/biochar production", as well as in Paper II (with regard to agronomic quality), where we tested biochar quality and emissions of gases and aerosols produced from various kilns (paper I) and its effect on maize crop growth (paper II).

Various feedstock and pyrolysis technologies can be used to produce a biochar that may result in different properties, which in turn impact the effectiveness of biochar on soil fertility (Butnan et al., 2015; Manyà, 2012). Various kiln types using low temperature pyrolysis (300-500 °C) were found to increase biochar yield and carbon content. By contrast, high temperature pyrolysis (>500 °C) has shown lower biochar yield with higher surface area and adsorption capacities for various compounds (Manyà, 2012). To the best of our knowledge, there has been no study published on the effect of kiln type (pyrolysis technology) on soil fertility and crop production.

So far, the agronomic effectiveness of biochar has been investigated where biochar was combined with either inorganic fertilizers or organic nutrients that were mixed separately in the soil either in the field or pot trial. Recently, techniques of biochar nutrient enrichment i.e. mixing nutrients with biochar before addition to the soil have resulted in significant improvement of crop yield (Schmidt et al., 2015). However, few studies exist where the agronomic effect of biochar enriched with mineral nutrients has been investigated. In addition, systematic studies on the optimal way to carry out such nutrient enrichments are lacking, certainly for mineral NPK.

It was hypothesized (hypothesis II-1) that biochar produced from various kilns with different pyrolysis conditions exhibits different crop yield effects depending on kiln type. It was further hypothesized (hypothesis II-2) that the effect of biochar on crop production is more pronounced when biochar is enriched with nutrients. These hypotheses were tested in Paper II entitled " Biochar from "Kon Tiki" flame curtain and other Kilns: Effects of Nutrient Enrichment and Kiln Type on Crop Yield and Soil Chemistry". Here, the agronomic effect of biochar produced from different kiln types and enriched in different ways (hot and cold enriched where hot and cooled-down biochar, respectively, were enriched with mineral fertilizers (NPK) dissolved in water and non-enriched biochar where the same amount was added separately) was investigated.

Promising agronomic effects of biochar addition have been found in wide range of latitudes when applied in low productive soils (Biederman & Harpole, 2013; Liu et al. 2013), mainly as a result of improved soil physicochemical or biological properties as described above. However, there is a knowledge gap regarding to what mechanisms can explain the positive effect of biochar on crop growth in particular soils.

In Nepal, soils are often moderately acidic showing low nitrogen (N), phosphorous (P) and exchangeable base concentrations (Schreier et al., 1994), which have adverse effects on soil fertility and crop production (Brown et al., 1999). However, thus far, few studies have been published where attempts were made to explicitly unravel the soil physical (Martinsen et al., 2014) and chemical mechanisms (Jeffery et al., 2017) responsible for the positive effect of biochar on crop production.

For the silty loam inceptisol studied, it was hypothesized that biochar alleviates moisture stress through enhanced soil water retention, thus increasing plant growth (Hypothesis III-1). It was also hypothesized that biochar alleviates nutrient stress by increased in-situ nutrient availability (associated with increased CEC) and by the direct addition of nutrients, especially K, common in the ash-component of biochar (Hypothesis III-2). It was further hypothesized (Hypothesis III-3) that biochar alleviates acid stress and thus increases plant growth by increasing soil pH, Ca/Al ratios and available P. These hypotheses were tested in Paper III entitled "Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil", where we investigated the effectiveness of biochar in alleviating these limitations (water, nutrient and acid stress) under maize plantation under controlled greenhouse conditions.

Many studies, thus far, have focused on the agronomic effect of biochar combined with inorganic fertilizers and less so on the effect of biochar combined with organic nutrients (co-compost). In addition to improved soil physicochemical properties (soil moisture, pH, CEC and exchangeable base cations) (Agegnehu et al., 2016) and increased available nutrients such as NO_3^-, P-AL and K (Kammann et al., 2015), the organic coating on the biochar particles formed under co-composting has been found to improve the soil redox (Eh) status (Husson, 2013). However, there are few studies, which have conducted systematic work to investigate the effect on soil quality and crop production of co-composted biochar, obtained through various composting methods.

Aerobic composting is common in most of the scientific studies where compost or co-compost matured with frequent turning of the piles (Hagemann et al. 2017). Effects on crop yield have been positive (Kammann et al., 2015). Another method is conventional composting, as traditionally done by farmers, involving maturation without turning the piles (Misra et al., 2003). Bokashi fermentation is third type composting (anaerobic lactic fermentation), which uses lacto bacilli bacteria (facultative anaerobe) to convert sugar into lactic acid that results in increased available nutrients and crop yield (Andreev et al., 2016; Dou et al., 2012). However, limited scientific research exists on the agronomic effect of lactic fermented bokashi-biochar mixtures. In addition, as far as we know, no study has been conducted where agronomic and nutrient effect (organic nutrient transformations) of co-

composted biochar and post mixed biochar-compost mixtures produced from these three composting processes (aerobic, conventional and bokashi) were explored.

It was hypothesized that biochar-compost mixtures especially co-composted biochar when compared with inorganic treatments and compost alone could 1) enhance soil available nutrients (mainly P and K) (Hypothesis IV-1); and 2) increase maize biomass growth as a result of the increased soil nutrient availability (Hypothesis IV-2). These hypotheses were tested in Paper IV entitled "Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth", where we tested the agronomic effect of co-composted biochar produced from three composting types (conventional, aerobic and Bokashi) on soil fertility and maize growth under controlled greenhouse conditions.

Co-composting with biochar has been found to increase nutrient retention capacity and increase crop production (Kammann et al., 2015). However, co-composting may not be feasible in all cases and thus, there remains the need to investigate the application of sustainable "raw" biochar obtained from common farmers field conditions. The majority of the studies have been done for one cropping cycle only, and few studies exist of long-term agronomic effects of biochar amendment (Griffin et al., 2017). Aged biochar has been found more effective in nutrient capture and delivery than fresh biochar (Haider et al., 2016), which may have long-term beneficial effect on crop production (Major et al., 2010).

In Nepal, average landholding size is low and the soils are often acidic and low in fertility (Brown et al., 1999; Schreier et al., 1994), which has severely affected the status of crop production over time (Shrestha and Pandit, 2017). Biochar amendment can be one alternative to improve soil fertility and farm production in a sustainable manner. In Nepal, there are very few studies where agronomic effect of raw biochar has been explored and none of the studies has executed agronomic trials for more than one cropping season. It is also necessary to investigate the appropriate biochar dosage that is economically and environmentally viable to be practiced by farmers under normal growing conditions.

In this study, we hypothesized (Hypothesis V-1) that the improved nutrient availability upon biochar addition increases crop yield for at least three cropping cycles (six cropping seasons) under field conditions. We further hypothesized (Hypothesis V-2) that the use of biochar can improve farming economics for small-scale farming in Nepal. These hypotheses were tested in Paper V entitled " Multi-year double cropping biochar field trials in Nepal: finding the optimal dosage through agronomic trials and cost-benefit analysis ", where we studied the effectiveness of six different dosages of biochar addition on soil fertility and crop production in small-scale agriculture. Furthermore, a cost-benefit analysis of biochar in small-scale agriculture was explored taking into account various carbon prices.

2. Materials and Methods

2.1. General approach of the trials

In this study, both greenhouse experiments and field experiments were conducted to assess the agronomic effect of biochar on moderately acidic silty loam soil from Rasuwa, Nepal. Greenhouse experiments were conducted in Kathmandu, Nepal to assess the mechanism of soil fertility effects of biochar especially nutrient enriched biochar and co-composted biochar. Also biochar production technologies were tested at this site. Field experiments were carried out in Rasuwa district, Nepal on the same soil, for three cropping years (six cropping seasons; two alternating crops) to investigate the long-term agronomic effect of biochar applied at six different dosages and find the optimal biochar dosage from an agronomic and socio-economic perspective.

For both the greenhouse and field trials, numerous laboratory analyses were carried out on soils with and without biochar amendment, and before and after aging in the field. The change in soil parameters upon biochar amendment was used to gather information about the working mechanisms of biochar for improved soil fertility.

2.2. Biochar production technology (Paper I)

Biochar was produced from Eupatorium feedstock using Flame curtain Kon-Tiki kiln with four different sub-types (deep cone metal kiln, small steel cone kiln, metal-shield soil pit kiln and soil pit kiln). Other feedstock blends (mixtures of wood, eupatorium shrubs, and rice husks) were also included producing between 120 to 800 l biochar per run. The differences between these different flame curtain sub-types were the diameter, the outer angle and the material of the kiln.

2.2.1. Principle of the flame curtain kiln

During the operational phase, at first, fire is started in the kiln, and the burning embers spread to form a first layer on the bottom of the kiln. A thin layer of biomass is then added on top of the embers, heats quickly and starts outgassing. The rising pyrolysis gas is caught in the flames and reacts with combustion air entering the kiln from the top. When ash appears on the outside of the carbonizing biomass, the next layer of biomass is homogenously spread on top. Convective and radiant energy from the flames above and from the hot pyrolyzing layers below heat the fresh biomass layer, which starts to pyrolyze (Schmidt et al., 2015). The biochar below the upper pyrolysis layer is shielded from oxygen access by the fire curtain itself. The combustion zone thus forms a flame curtain that protects the underlying biochar from oxidizing and cleanly burns all pyrolysis smoke and gases (Fig.16b) as they pass through this hot fire front. The temperature in the main pyrolysis zone just below the flame curtain is 680°C to 750°C. The manual layering of biomass is repeated until the metal kiln or soil pit is filled (Fig.16d) and the pyrolysis process is ended either quenching with water or soil (Fig.16e).

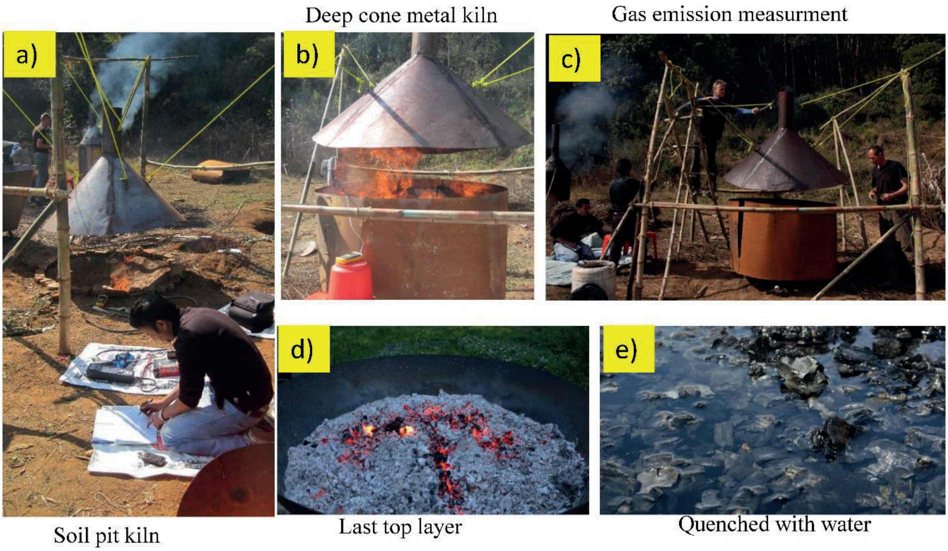


Fig 16. Flame curtain kiln (soil pit and deed cone metal kiln) operation at Matatirtha, Kathmandu, Nepal

2.2.2. Gas and aerosol emission factors

The principle of establishing these emission factors was to determine a carbon balance for the pyrolysis processes in the various kilns. For such a carbon balance, the amount of C entering the system with the feedstock is compared to the amount of C in the end product (biochar). The difference is the amount of C emitted during the pyrolysis. By measuring all C-containing gases and aerosols, a carbon balance can be established and emission factors per kg of biochar can be calculated (Pennise et al., 2001; Sparrevik et al., 2015).

Gas emissions were measured and analyzed for CO_2, CH_4, non-methane volatile organic carbon (NMVOC), nitric oxides (NO_x) and aerosols (total suspended particles, TSP, derived from PM_{10}. A

Microtector II 6460 was used to analyze carbon dioxide (CO_2) and methane (CH_4). Carbon monoxide (CO) and nitric oxide (NO) were analyzed with a Kigaz 300 flue gas analyzer by internal jacket type electrochemical sensors. Particles in the form of PM_{10} were analyzed with a Thermo Scientific pdr-1500 instrument by use of photometric detection of particles (detection limit 0.1 μg/m^3). For conversion of concentration from mass units to molar ratios in the particle measurements, all particles were assumed to consist of elementary carbon.

2.2.3. Biochar characterization

All biochar were characterized for CEC by extraction with ammonium nitrate (1M NH_4NO_3) and the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Carbon content in feedstock and biochar was measured in triplicates on 100-mg samples that were combusted at 1030 °C and analyzed in an element analyzer (Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)). Three biochars representing two different kiln types (soil pit kiln and metal cone kiln each 70° - 1m50 diameter) and two feedstock (100% Eupatorium and 50:50 Eupatorium : hard wood) were analyzed by a EBC accredited laboratory following the EBC certification program and methods (EBC, 2012). Five example biochars were further analyzed for 15 individual PAHs by 36-h exhaustive toluene Soxhlet extraction according to published procedures (Hale et al., 2012) and surface area by N_2 adsorption at 77 K.

2.3. Greenhouse experiment (Paper II, III and IV)

2.3.1. Overview of the pot trial

Soil used under pot trial was collected from agricultural land, Rasuwa district, Nepal (N 28^0 00', E 85^0 10'). The sampled soil used in this experiment was moderately acidic (pH_{CaCl2} 4.5; pH_{water} 5.1), low-CEC (6.05 $cmol_c$ kg^{-1}), silty loam (Table 2). Pots (top, middle and bottom diameter: 24 cm, 19 cm and 12 cm respectively; height 20 cm; volume 6 L) were filled with 3 kg air-dried soil. Biochar produced from *Eupatorium* feedstock via soil pit flame curtain kiln was used in the greenhouse experiment. Maize crop (*variety; Manakamana*) was planted under greenhouse experiment (Fig.17) Maize seed was sown 2 cm below the soil surface in each pot. Maize plants were harvested at 7-8 weeks' after sowing and the maize aboveground biomass was oven dried at 70 ^{0}C (24 h) for dry weight measurement. Soil from all individual replicate pots were collected to make a composite sample after harvesting maize plants.

Greenhouse

Maize grown in greenhouse experiment

Fig 17. Maize planted in controlled greenhouse conditions at Matatirtha, Kathmandu, Nepal

Table 2. Properties of biochar and soil used in greenhouse and field experiment.

Properties	**Biochar**	**Soil**[1]
pH_{CaCl2}	9.3	4.5
pH_{H2O}	-	5.1
CEC ($cmol_c\ kg^{-1}$)	72	6
BS (%)	-	51
Ca^{2+} ($cmol_c\ kg^{-1}$)	18	2.3
Mg^{2+} ($cmol_c\ kg^{-1}$)	13	0.56
K^{+} ($cmol_c\ kg^{-1}$)	36	0.20
Al^{3+} ($cmol_c\ kg^{-1}$)	-	1.6
Ca/Al ratio	-	1.4
Total organic C %	70	1.6
Total H %	1.1	1.02
Total N %	0.46	0.13
Total P($g\ kg^{-1}$)	-	-
Total K ($g\ kg^{-1}$)	-	-
Available P $mg\ kg^{-1}$	-	12
Surface area	74.6	-
Textural class	-	Silty loam[2]
Order	-	Inceptisols

[1] Soil test before operating field trial experiment

[2] Silty loam with 33% sand, 50% silt and 17% clay

2.3.2. Kiln type and nutrient enriched biochar experiment (paper II)

In addition to flame curtain kiln (four sub types; deep-cone metal kiln, steel-shielded soil pit, conical soil pit and steel small cone kiln), biochars made in three other kiln types (traditional brick kiln, earth mound kiln and TLUD kiln) were used in the greenhouse experiment. Biochar was nutrient enriched using two methods, namely hot and cold nutrient enrichment. Hot and cold nutrient enrichment refers to hot and cooled-down biochar, respectively, that were enriched with mineral fertilizers (NPK) added dissolved in water (Fig.18). The nutrient solution contained urea, di-ammonium phosphate (DAP) and potash as the source of nitrogen (2.7 g pot^{-1}), phosphorous (1.08 g pot^{-1}) and potassium (1.08 g pot^{-1}) respectively. Hot nutrient enrichment was carried out by pouring hot (200 to 400 ^{0}C) biochar at the rate of 30 g and 120 g (equivalent to 1% (20 t ha^{-1}) and 4% (80 t ha^{-1} biochar respectively) in 1 L dissolved nutrients in a bucket (Fig.18, middle image). The lukewarm mixture in the bucket was then stirred thoroughly for 10 minutes to ensure that biochar was well mixed with the solution. Cold nutrient enrichment was carried out using a similar method with the same volume of water and amount of NPK but adding biochar that was water quenched and cooled down beforehand.

Weighing hot biochar — Hot or cold nutrient enrichment — Non-enrichment (biochar and NPK added separately)

Fig 18. Nutrient enrichment techniques of biochar

A pot trial was carried out to investigate the effect of different biochars, produced from various kiln and enriched in different ways (hot nutrient-enriched, cold nutrient-enriched and non-enriched biochar) on soil characteristics and crop production. The pot trial was carried out in June-July 2015. Various kiln type and nutrient enriched biochar comprised of 21 treatments including two control treatments (fertilized control and non-fertilized control) with five replications (n=5) arranged in randomized complete block design.

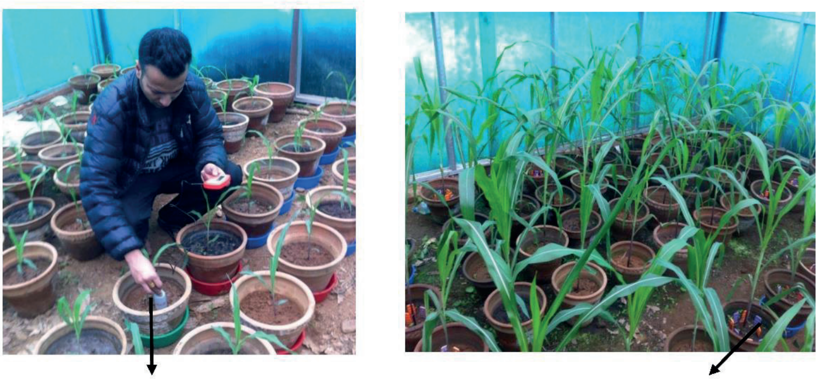
2.3.3. Mechanism of Biochar: water, nutrient and acid stress alleviation experiment (paper III)

A pot trial was carried out from 11th May to 5th July 2016. The effects of biochar on the alleviation of three potential physical-chemical soil limitations for maize growth were investigated, i.e. water stress, nutrient stress and acid stress. Experiments involved soils with two dosages of biochar (0.5% and 2% w:w), as well as ones without biochar, in combination with four different dosages of water, NPK fertilizer and lime under water stress, nutrient stress and acid stress experiment respectively (Table 3). Each set of experiments comprised nine treatments with four replications each in completely randomized design (n=4) resulting in 36 pots per experiment. Three treatments receiving the highest amount of water (200 ml per pot per day), NPK (1.17 g per pot) and lime (4.7 g per pot) were added that were considered as shared (common) treatments for each of the water stress, nutrient stress and acid stress sets of experiments.

Table 3. Amount of water, NPK and lime used in water stress, nutrient stress and acid stress experiment.

Water stress experiment	**Nutrient stress experiment**	**Acid stress experiment**
20% water (40 ml water per pot per d)	No NPK	No lime
40% water (80 ml water per pot per d)	1/3rd NPK (0.39 g NPK per pot)	0.25g lime per pot
70% water (140 ml water per pot per d)	2/3rd NPK (0.78 g NPK per pot)	0.75 g lime per pot
Full water (200 ml water per pot per d)	Full NPK (1.17 g NPK per pot)	4.5 g lime per pot

In-situ soil moisture content (% vol.) was measured in the water stress alleviation treatments only, using a hand-held Time-Domain Reflectometer (TDR) just before each irrigation (Fig.19, *left image*). Likewise, in-situ availability of cations and anions in the soil were measured using plant root simulator probes (PRS™; Western Ag, Saskatoon, Canada) from nutrient stress and acid stress experiment (eight treatments with three replications in total; Fig.19, *right image*).



Soil moisture content measured with TDR

Plant root simulators (PRS ™ probes) for in-situ nutrient supply rates

Fig 19. Soil moisture content (*left image*) and plant root simulators in-situ nutrient supply measurement (*right image*) under water stress and nutrient stress experiment respectively.

2.3.4. Co-composted biochar experiment (Paper IV)

In this study, we focused on three different composting methods (Fig.20) to provide nutrients from raw materials (green waste and farmyard manure in the ratio 1:1.5w/w wet weight): i) conventional composting (maturation without turning the piles), ii) aerobic composting (frequent turning) and iii) bokashi composting (anaerobic lacto-fermentation). Composting was carried out in the absence (compost alone) and presence of biochar (co-composting). All three composting process lasted for 80 d (11th July - 29th September, 2016).

Conventional and aerobic composting methods

Bokashi fermentation process

Fig 20. Conventional and aerobic composting (*left image*) and bokashi fermentation (*right image*) carried out in Nepal.

All three types of compost, co-compost and post mixed biochar-compost mixtures (biochar mixed with three compost types separately just before the experiment) were used at two different dosages (40 g per pot and 120 g per pot equivalent to 20 t ha^{-1} and 60 t ha^{-1}), illustrating 18 treatments in total. In addition to these, four additional treatments were tested; (1) NPK equivalent to available nutrient content supplied by 20 t ha^{-1} of composts (0.12 g N, 0.06 g P_2O_5 and 0.24 g K_2O), (2) NPK equivalent to available nutrient content supplied by 60 t ha^{-1} of composts (0.36 g N, 0.18 g P_2O_5 and 0.72 g K_2O), (3) NPK equivalent to available nutrient content supplied by 20 t ha^{-1} of composts + 3 g biochar and (4) NPK equivalent to available nutrient content supplied by 60 t ha^{-1} of composts + 9 g biochar). The amount of NPK content in 20 t ha^{-1} and 60 t ha^{-1} compost was calculated by assuming a 15% N availability, 30% P and K availability in the compost (Kammann et al., 2016). In total, 22 treatments with four replications (n=4) were arranged in completely randomized design. Pot trial was started from 12th October and lasted for 55 d.

2.3.5. Multi-year double cropping biochar field trials (paper V)

A multiblock repeated controlled field trial was established in rainfed uplands on the private farmland located at Dhaibung VDC, Rasuwa district, Nepal (28^0 00' N, 85^0 10'E) receiving average annual rainfall of 1850 mm and mean annual temperature of 15.4 °C. Soils are moderately acidic and have low CEC (Table 2). Biochar was produced from Eupatorium using traditional earth mound kiln with pyrolysis temperature of 450 - 500^0C. Traditional soil pit kiln was used as the flame curtain kiln had not been developed in Nepal when the field trial was established. Twenty-four plots of 10 m^2 each were established on a flat area without shading trees with 1 m spacing between plots. Six treatments with four replications (n=4) were assigned in completely randomized design (CRD) where six different dosages of biochar (0, 5, 10, 15, 25 and 40 t ha^{-1}) were deployed. Higher dosages (25 t ha^{-1} and 40 t ha^{-1} biochar) are little realistic and are included only for scientific reasons. All treatments including control received equal amounts of mineral fertilizer N (in the form of urea; 60 kg N ha^{-1} after 60 d) and farmyard manure (a composted mixture of cow manure and greenwaste, 30 t ha^{-1} wet weight) according to farmers practice. During land preparation, biochar and manure were spread evenly followed by tillage (15 cm soil depth) and harrowing practices in all treatment plots (Fig.21). After a week of land preparation, maize seed (*Arun variety*) was sown at a depth of 5-6 cm following 30 cm x 30 cm spacing within each treatment plot. The field trial was set up in April 2014. Each year, maize was grown in the wet season (April to August) followed by mustard in the dry season (September to February). This cropping pattern (maize-mustard) was continued for three years (until February 2017). Biochar was applied only once at the onset of the trials (April 2014).

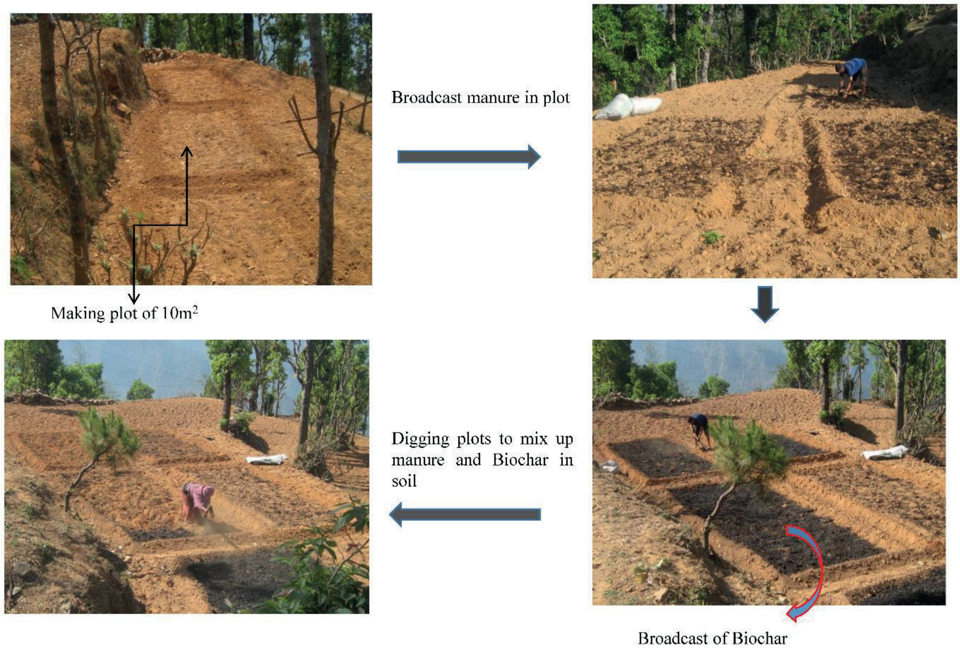


Fig 21. Land preparation of agronomic field experiment plot, Rasuwa, Nepal.

Upon harvest (each year), air-dried grain yield of both the crop were measured. After 2.5 year (5th season), soil sample from all treatment plots were assembled to make a composite soil sample and were analyzed for pH, CEC, total CHN, P-AL and PAW. In addition, with a view to explore the stability of the biochar under field conditions, soil from the control and 40 t ha^{-1} field aged biochar plots, along with the fresh non-aged biochar, were subjected to BPCA analysis.

Cost-Benefit analysis of biochar farming applied at six different dosages for three subsequent year (year 2014 to year 2017) under maize and mustard-cropping system was calculated on the basis of the agronomic results obtained. The agronomic cost included farm inputs such as seeds, urea, manure and labor for land preparation. Biochar production cost included labor for kiln construction and operation as well as health cost of gas (CO) and aerosol (smoke, $PM_{2.5}$) emissions during biochar making (analogous to Sparrevik et al. (2014)), in addition to climate cost of CH_4 emissions (taking into account the 27-fold higher global warming potential of CH_4 as compared to CO_2) during biochar making (Smebye et al., 2017; Sparrevik et al., 2014). The gas emissions from soil pit flame curtain kiln were used for the cost-benefit analysis as this novel method is the one of choice in practice and far preferable over traditional kilns, due to low gas and aerosol emissions, as well as easy and quick operation (paper I). However, financial cost of biochar making, agronomic effect of resulting biochar's, as well as methane emissions, would have been similar for traditional and flame curtain kilns. Only CO emissions and resulting health effects would have been higher for traditional kilns. Thus, the outcome of the cost-benefit analysis would have been almost the same for traditional kilns (gross margins being maximally 5% lower, with the same trends between C price scenarios and biochar dosages). Income was calculated from crop sale (both maize and mustard) and possible carbon sequestration benefits at various C prices. Gross margin/profit of biochar-inclusive farming (Total income - Total cost) was calculated as a function of biochar dosage, assuming a medium social cost of carbon (SSC), of US$42 per ton CO_2 (SCC at 3% discount rate and emitted in 2020) (EPA, 2013). In addition, various carbon prices were used, ranging from no carbon price (US$0 per ton CO_2), as would be the current situation, to current voluntary carbon market prices (US$6 per ton CO_2), and a high-impact SSC of US$147 per ton CO_2 (EPA, 2013).

2.4. Soil analysis

Soil from all individual replicate pots were collected to make a composite sample for each of treatments. Soil samples were analyzed for pH, Eh, CEC, total carbon, hydrogen and nitrogen (CHN), plant available P (P.AL), plant available water (PAW). Soil pH was measured in both 0.01M $CaCl_2$ and in water (soil : solution ratio of 1:2.5 in volume basis) using an Orion 1 Ross pH electrode. Soil redox potential (Eh) was measured with WTW equipment with AgCl reference electrode (combined 3M AgCl electrode) and corrected to standard hydrogen electrode (SHE) as a function of temperature. For CEC, soil was extracted with 1M NH_4NO_3 and the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}) were measured in the leachates using inductively coupled plasma optical emission spectrometry (ICP-OES). Exchangeable H^+ was determined by titration with 0.02 M NaOH to pH 7. Sieved samples were crushed for total CHN analysis with a CHN analyzer (LECO, Truspec). Plant available phosphorous (P-AL) was measured by the ammonium lactate method (Krogstad et al., 2008) where 40 ml of ammonium lactate solution was added to 2 g dry soil (sieved < 2 mm) and shaken in a rotating shaker (1.5 hours), and filtered, (0.45 µm). Ascorbic acid (0.4 ml) and molybdenum reagent (0.4 ml) was added to both standard solution and the extracted soil samples and measurements were done using a spectrophotometer (Gilford Stasar Spectrophotometer) at 700 nm. For PAW measurement, hand-packed soil samples were saturated and soil water measured at different matrix potentials (pF 2, field capacity and pF 4.2, wilting point) through ceramic pressure plates (Obia et al., 2016). PAW (% vol.) was calculated as the difference between field capacity (% vol.) and wilting point (% vol.). BPCA analysis was performed following the methods of Brodowski et al. (2005) and Dittmar (2008) with modifications. Briefly, samples were digested in 4 M trifluoroacetic acid (TFA, 105°C, 4 h) to remove metals and polyvalent cations. Residue were then extracted in 0.5 mL of 65% HNO_3 at 170°C for 8 h under high pressure, then purified using Dowex cation exchange resin (50W, 200–400 mesh). Finally, BPCA compounds (B3CA to B6CA with 3 to 6 carboxyl group substituents, respectively) were identified via HPLC-DAD using certified standards.

2.5. Statistical analysis

All data were analyzed using R statistical software version 3.2.2. Normality and homogenous variances of all data sets were tested with Sharpio-Wilk -and Levene's test, respectively. Two sample t-test was operated to compare the significant differences between two groups or treatment means (Paper I). Data were analyzed through ANOVA model to explore the effect of independent explanatory categorical variable on the dependent response variable followed by subsequent post hoc Tukey test ($p<0.05$) to find out the differences of treatment means. Post hoc *REG-WQ* test was used in paper IV. In addition to ANOVA, an ANCOVA model (combination of categorical and continuous variable) was also used in paper III to find out if there is any confounding variables (covariates) associated with categorical variable to explain the crop yield effect. Regression model was used to find the relationship (correlation) between two continuous variables to explain the model.

3. Main results and discussion

3.1. Paper I. Biochar properties and gas emission during biochar production

3.1.1. Biochar yields and properties

Average biochar yields from *Eupatorium* feedstock using four different types of flame curtain were 21 ± 3 % on a dry weight basis and 37 ± 5 % on a C basis (Table 4). This is in the same order of magnitude as other high temperature (700°C) pyrolysis systems (Chen et al., 2015; Mašek et al., 2013). Yields were better than those of traditional low-temperature kilns but lower than those of low- temperature retort kilns (typically around 30-40% on a dry weight basis (Sparrevik et al., 2015)).

The average carbon content of biochar was 77 ± 3 % (Table 4). CEC was high, ranging from 55 cmolc kg^{-1} (steel shielded soil pit) to 121 cmolc kg^{-1} (steel deep cone), with one char even showing CEC above 200 cmolc/kg, which is on the high end of literature values for field-made biochars (Lehmann and Joseph, 2015; Martinsen et al., 2015). Surface area of biochar was 89 ± 33 $m^2\ g^{-1}$ (ranging from 35 to 111 $m^2\ g^{-1}$) (Table 4), which is in agreement with other biochars produced with industrial technology at temperatures of 600° to 750° C (Mukherjee et al., 2011). The most toxic compound among the PAH-16 used as benchmarks by the environmental authorities in many countries is benzo (a) pyrene (BaP). Concentrations of BaP were 0.01-0.04 mg kg^{-1} (Table 4), well below the Norwegian maximum tolerable risk (MTR) level for soils where 95% of art diversity is protected (0.5 mg kg^{-1}) (Bakke et al., 2007). In addition, PAHs in biochar are only very sparingly bioavailable, often less than 1% (Hale et al., 2012). PAH EPA16 contents were low (2 to 4.4 mg kg^{-1}) (Table 4) most probably due to the optimized out-gassing under the fire front.

Table 4. Characterization of biochar produced from Eupatorium feedstock using four subtypes of flame curtain kiln

Kiln type	**Biochar yield and properties**						
	OC	**yield**	**C yield**	**CEC (washed)**	**SA**	**PAH**	**BaP**
	%	**%**	**%**	**cmolc kg^{-1}**	**$m^2\ g^{-1}$**	**mg kg^{-1}**	**mg kg^{-1}**
Steel deep-cone	77	19	36	121	84.9	3.7	0.016
Steel shielded soil pit	71.2	25	44	55	35.4	1.9	0.013
Conical soil pit	71.7	18	31	68	111	2	0.037
Steel small cone	70.8	21	35	112	99.56	4.4	0.039

3.1.2. Emission factors

Emission factors were, in g per kg biochar produced: CO 54 ± 35, CH_4 30 ± 59, TSP 11 ± 15, NMVOC 6 ± 3, NO_x 0.4 ± 0.3, and total products of incomplete combustion, PIC, 100 ± 83 (Fig.22). These data are based on 17 runs of 10 to 15 data points each, totaling around 250 individual measurements per gas/aerosol. Retort kiln values (5 runs) were taken from (Sparrevik et al., 2015) and traditional kiln (8 runs) from (Pennise et al., 2001; Sparrevik et al., 2015), because these measurements were carried out with exactly the same equipment and measuring methodology.

The flame curtain kilns had significantly lower emissions of CO, NO_x, total products of incomplete combustion (PIC) than non-retort (traditional) or retort kilns (Fig.22). A similar trend was observed for Non-methane volatile organic carbon (NMVOC) emissions, where emissions were significantly lower for flame curtain kilns (6 ± 3 g kg^{-1} biochar) than for non-retort traditional kilns (53 ± 4 g kg^{-1} biochar) (Fig.22). However, no significant differences were revealed for methane and TSP emissions between flame curtain, retort and traditional kilns. CO_2 emissions were significantly higher for the flame curtain kilns than for retort or traditional kilns, which is a direct consequence of the slightly lower yields and lower non CO_2-emissions obtained in flame curtain kilns. CO_2 is the lowest caloric and least climate hazardous emission product of biomass combustion and a measure of the completeness of the combustion of pyrolytic gases.

Pyrolysis temperatures of the flame curtain kilns (700 °C) are higher than those of traditional or retort technologies (400-500 °C) (Pennise et al., 2001; Sparrevik et al., 2015), and this results in a more porous and more condensed biochar (Lehmann, 2007b). Higher porosity certainly implies stronger contaminant immobilization (Hale et al., 2016) and probably also higher nutrient retention (Kammann et al., 2015). More condensed higher-temperature biochars exhibit higher H/C_{org} ratios which have been related to stronger N_2O emissions reductions upon their amendment to soil in a recent meta-analysis (Cayuela et al., 2014). In another meta-analysis higher-temperature chars have tentatively been associated with negative priming, i.e., increases in soil organic matter upon the amendment of biochar to soil (Zhang et al., 2013). Overall, in many cases the high-temperature flame curtain chars can be expected to be of higher quality than lower-temperature ones made by traditional technologies, depending on the purpose the respective biochar or charcoal.

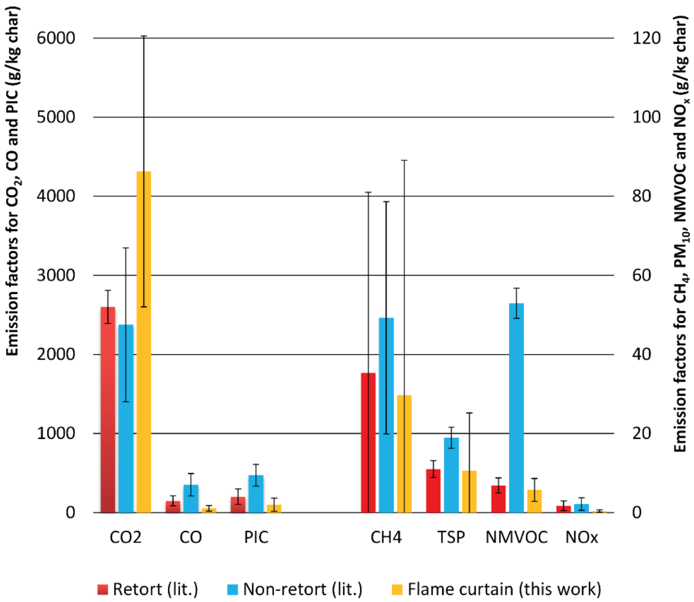


Fig 22. Emission factors for CO_2, CO, CH_4, TSP (aerosols, derived from PM_{10} as described in the methods, non-methane volatile organic carbon (NMVOC), and the sum of nitrogen oxide and nitrogen dioxide (NO_x), as well as the sum of all products of incomplete combustion, PIC (all C-containing gases except CO_2). Flame curtain: based on 17 runs of 10 to 15 measurements each done within the present study. Retort and non-retort kilns: average values from refs (Pennise et al., 2001; Sparrevik et al., 2015). Error bars represent standard deviations in 50 to 250 individual measurements.

3.1.3. Implications

Operation of medium sized kiln have both pros and cons in terms of cost, emissions, use and other socio-economic benefits (Table 5). However, the important challenge is to identify the most economical (low cost) and ecofriendly technology (low emission). Among various kilns explored in this study, flame curtain kiln offers multiple advantages such as low cost, easy operation, feasibility for small scale farmers, low gas and aerosols emissions, no start up wood required compared with retort kiln and a good quality biochar harvested in relatively short time (3 hours for 1 m^3 biochar) (Table 5). The biochar yield of 21 ± 3 %, which is somewhat lower than that of retort kilns (Adam, 2009; Sparrevik et al., 2014) is a disadvantage of flame curtain. However, this is a modest limitation in the case of biochar for soil amendment made from low value organic waste such shrubs, straw and husks that cannot be pyrolysed in such retort system without a large portion of valuable fire wood. This could be an important factor to consider in the case of charcoal making from high-value wood for cooking purposes, where yields need to be high in order to reduce deforestation and increase the economic value of the charcoal making activity.

Table.5. Advantage and disadvantage of various medium sized kilns

Kiln type	**Application**	**Main advantages**	**Main disadvantages**
TLUD	Kitchen gardens, cooking purposes	• Energy for cooking • Saving firewood • Low gas emission factors	• Too small to generate larger amounts of biochar
Traditional kilns	Agriculture, charcoal making	• Familiarity • Low investment cost • Complete pyrolysis of thicker logs	• High gas emission factors • Slow (4 days)
Retort kilns	Agriculture (possibly + energy), charcoal/briquette making	• Lower emissions than traditional kilns • High biochar yield • Energy generation possible with pyrolysis heat • Complete pyrolysis of thicker logs	• High investment cost • Startup wood required • Complicated construction and operation • Slow (2 days)
Flame Curtain Kilns	Agriculture + heat, charcoal making (small logs)	• Relatively low emissions esp. of CO • No startup wood required • Easy to construct and operate • Fast (3 hours for 1 m^3 biochar) • Low to zero investment cost • Heat recovery	• Relatively low biochar yield (charcoal making) • Incomplete pyrolysis of thick logs
Power-generating systems	Energy + agriculture, briquette making	• Power generation • Negligible emissions	• Relatively high investment cost • Low caloric content of briquettes

In conclusion, good quality biochar with much carbon retained, high CEC, surface area and low PAH were produced from flame curtain kilns (Table 4). In addition, the emission factors were significantly lower than those of traditional and retort technologies (Fig.22). Thus, the hypothesis was accepted that biochar produced from Eupatorium feedstock using flame curtain "kon-tiki" kiln produces biochar of good quality with low gas and particle emissions.

3.2 Paper II. Effect of kiln type and mineral nutrient enriched biochar on crop production

3.2.1. Effect of kiln type biochar on biomass production

Amendment with biochar produced from seven different kiln types did not show significant variation in maize biomass production (aboveground biomass (AGB), height and node diameter) (Table 6). The agronomic effect of the flame curtain kiln biochar was similar to that of the other kiln types. Thus, hypothesis II-1 that the biochar from various kiln has different crop yield effects was not supported by our data and falsified. The finding was supported by the observation that kiln type did not reveal significant variation in biochar characteristics such as CEC, pH and OC content. Though, flame curtain kilns showed lower emission factors and higher biochar production efficiencies (Paper I, Cornelissen et al. (2016)), none of the four different flame curtain kilns differed in biochar properties and biomass production compared with other kilns. Similar non-significant trends of crop production with kiln type were observed for the biochar produced from ponderosa pine and macadamia nut feedstock under slow and fast pyrolysis types for perennial grass, *Koeleria macrantha* (Gundale and DeLuca, 2007) and lettuce/maize corn (Deenik et al., 2010), respectively. Furthermore, biochar produced from rice husk using traditional kiln type (slow pyrolysis) did not show significant effect on rice yield (Haefele et al., 2011). As we know, this is the first study that directly compared the agronomic effect of biochar produced by various kiln types.

Table 6. Statistical analysis of Two factor ANOVA (kiln type and mineral nutrient enrichment type's biochar) on maize biomass yield (N= 77).

	Maize dry AGB (g)		**Maize height (cm)**		**Maize node diameter (cm)**	
Factor	*f-value*	*P*	*f-value*	*P*	*f-value*	*P*
Kiln type	*1.2*	*> 0.1*	*1.4*	*> 0.1*	*2.3*	*> 0.05*
Nutrient enrichment	*123.4*	*< 0.0001*	*104.5*	*<0.0001*	*24.9*	*< 0.0001*
Kiln type and nutrient enrichment type	*7.5*	*< 0.001*	*3.5*	*< 0.01*	*1.3*	*> 0.01*

3.2.2. Effect of nutrient enriched biochar on maize biomass production

Nutrient enrichment, in contrast to kiln type, showed significant effects (*P<0.0001*) on maize biomass production (Table 6). Biochar hot nutrient enrichment at 1% dosage increased biomass by +153% and +209% of the values observed for 1% dosage cold nutrient-enriched and non-enriched biochar respectively (Fig.23). Similar trend was observed for 4% dosage hot nutrient enriched biochar, which showed higher (*P<0.001*) average biomass than the 4% non-enriched (+82%) and cold-enriched biochars (+62%) (Fig.23). Overall, hot nutrient enrichment showed better effects on biomass production than cold nutrient enrichment or non-enrichment (biochar and nutrient added separately). Thus, hypothesis II-2 that the biochar enrichment would have better crop yield was only supported by our data and was accepted with respect to hot, but not cold, mineral nutrient enrichment.

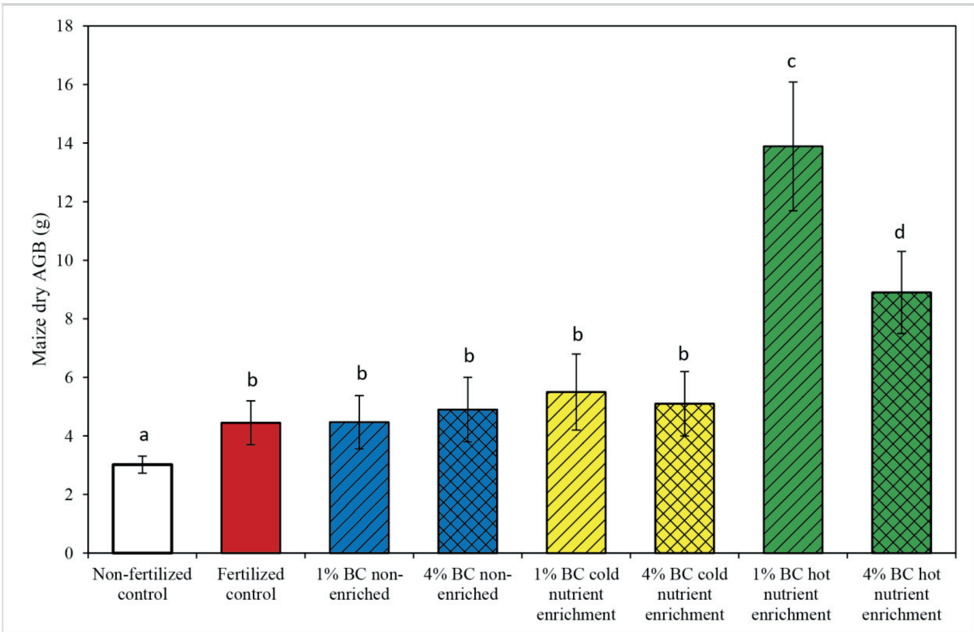


Fig 23. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize dry biomass production (g). Different letters above the bars represent significant differences between mineral nutrient enrichment types and controls

Nutrient enrichment could be an effective method to improve soil fertility because nutrients become reversibly trapped in the nano/micropores inside the biochar matrix where water movement is restricted, and act as a slow-release fertilizer, reducing nutrient leaching on low CEC soils (Alling et al., 2014; Hale et al., 2013). Schmidt et al. (2017) reported increased tea leaf yield by +300% and +250% upon both hot and cold urine enriched biochar compared to control and pure biochar respectively. Another study by Kammann et al. (2015) showed that organic nutrient enriched biochar (co-compost) increased crop production compared with control and pure biochar possibly due to enrichment of nutrient (nitrate and phosphate) in biochar pores. In our study, hot nutrient enrichment showed better agronomic performance over cold enrichment, which can possibly be explain by analogy with organic compound diffusion through soil and black carbon nanopores. The penetration of nutrients into biochar nanopores is most likely an activated process that probably takes place faster at increased temperatures: retarded nanopore diffusion of organic compounds is a highly activated process with activation enthalpies ranging from 60 to 100 kJ/mol (Cornelissen et al., 1997). This implies that the retarded pore diffusion rates, and thus the rates of nanopore penetration, increase by approximately a factor of 2 for each 10 °C increase in temperature (Cornelissen et al., 1997). Thus, we speculate that pore penetration in hot biochar (e.g., between 60 and 100 °C, the expected temperature range when 100-200 °C hot biochar is brought into water) could be 100-10,000 times faster than that at room temperature, analogous with observations for organic molecules in black carbon pores that showed 100 times faster diffusion at 60 °C than 20 °C (Hu and Wang, 2003; Werth and Reinhard, 1997). However, more research has to be undertaken to explain the underlying nutrient enrichment mechanisms, including nutrient speciation and location on the microscopic level (Agyarko-Mintah et al., 2016), and their effects on crop production.

3.4. Paper III. Effect of biochar in alleviating nutrient stress

3.4.1. Effect of biochar addition on soil properties

Biochar addition (2% w:w) significantly improved soil physio-chemical properties such as pH, CEC and total OC% as well as exchangeable K^+, Mg^{2+} and Ca^{2+} (Table 7).). Biochar addition significantly increased plant available phosphorous (P-AL) and water (PAW) in this soil. Similarly, biochar addition increased in-situ P and K supply as well as Ca/Al ratio but not mineral nitrogen (NO_3^-) as measured with PRS ™ probes (Table 7).

Table 7. Effect of biochar on soil physical and chemical properties. Treatments with different biochar dosages (0% BC or control, 0.5% and 2% BC) receiving highest amount of agricultural inputs (water, NPK and lime) i.e. the three shared/common treatments. Soil properties values are given as mean ± SD, n=3. Letters a, b and c denotes significant differences between biochar vs non-biochar (control) treatments on soil properties.

Properties	**Common treatments with full NPK, lime and watering rates**		
	0% BC (control)	**0.5% BC**	**2% BC**
Total Organic C%	1.35 ± 0.0 a	1.64 ± 0.01 b	2.94 ± 0.02 c
Total Nitrogen%	0.12 ± 0.01 a	0.12 ± 0.01 a	0.14 ± 0.01 a
Total Hydrogen%	0.48 ± 0.01 a	0.47 ± 0.01 a	0.48 ± 0.00 a
pH (0.01M $CaCl_2$)[a]	5.34 ± 0.15 a	5.87 ± 0.13 b	6.58 ± 0.13 c
CEC ($cmol_c\ kg^{-1}$)	7.63 ± 0.7 a	8.69 ± 0.45 a	11.92 ± 0.24 b
Ca^{2+} ($cmol_c\ kg^{-1}$)	5.96 ± 0.24 a	6.38 ± 0.24 a	8.87 ± 0.24 b
Mg^{2+} ($cmol_c\ kg^{-1}$)	0.54 ± 0.02 a	0.67 ± 0.01 b	1.07 ± 0.04 c
K^{+} ($cmol_c\ kg^{-1}$)	0.26 ± 0.02 a	0.55 ± 0.07 b	1.75 ± 0.12 c
Al^{3+} ($cmol_c\ kg^{-1}$)	0.03 ± 0.03 a	0.006 ± 0.00 a	0.006 ± 0.00 a
H^{+} ($cmol_c\ kg^{-1}$)	0.81 ± 0.84 ab	1.05 ± 0.19 a	0.17 ± 0.14 b
Sand %	32.70 ± 0.49	32.1 ± 0.35	32.70 ± 0.49
Silt %	49.90 ± 0.43	50.6 ± 0.55	50.70 ± 1.05
Clay %	17.40 ± 0.11	17.40 ± 0.37	16.70 ± 0.60
Textural class	Silty loam	Silty loam	Silty loam
Soil moisture content (% vol.)[b]	6.9 ± 0.6 a	19.1 ± 1.4 b	39.3 ± 2.1 c
Field capacity (% vol)	29.83 ± 1.83a	29.96 ± 1.34a	35.30 ± 0.18b
Plant available water (% vol)	20.82 ± 1.97a	21.18 ± 0.78a	25.55 ± 0.54b
P-AL ($mg\ kg^{-1}$)	11.10 ± 0.30 a	23.36 ± 0.28 b	84.16 ± 1.08 c
PRS™ adsorbed cations and anions			
NO_3^{-1} (µg per 10 cm^2)	304 ± 158 a	636 ± 131 a	783 ± 257 a
Ca^{2+} (µg per 10 cm^2)	1350 ± 386 a	2401 ± 645 b	2259 ± 99 b
Mg^{2+} (µg per 10 cm^2)	103 ± 45 a	223 ± 18 b	284 ± 30 b
K^{+} (µg per 10 cm^2)	41 ± 11 a	156 ± 29 b	384 ± 144 c
P (µg per 10 cm^2)	1.2 ± 0.4 a	3.1 ± 0.4 b	3.5 ± 3.3 b
Fe^{3+} (µg per 10 cm^2)	40 ± 23.7 a	103 ± 4 b	86 ± 27 b
Al^{3+} (µg per 10 cm^2)	31 ± 16.6 a	54 ± 16.8 a	24 ± 6.7 a
Ca/Al (molar ratio)	32.2 ± 9.0 a	32.3 ± 17.7 a	63.8 ± 18.6 b

[a] Soil pH was averaged and pooled for standard deviation from 1 d, 24 d and 50 d (in-situ and ex-situ pH measurement) to give one final reading (mean ± SD)

[b] Daily measured in-situ soil moisture percentage measurement (% vol.), n=50

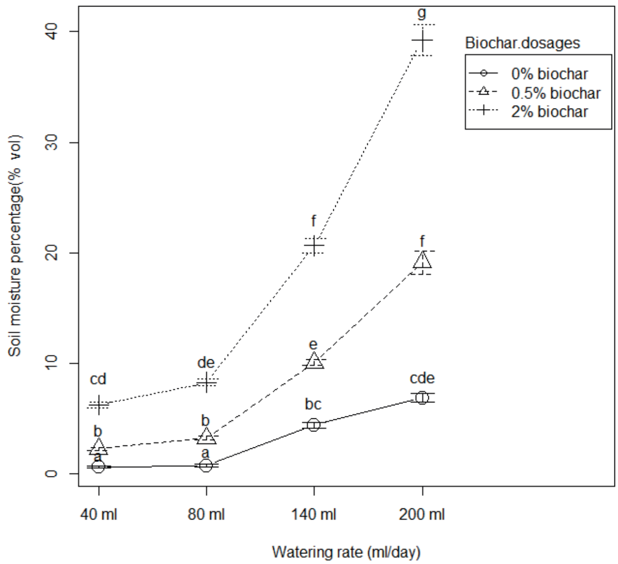


Fig 24. Effect of biochar dosages and watering rate on soil moisture content (percentage by volume). Soil moisture percentages measured at five-day intervals after second leaf emergence at 15 d until harvest at 50 d. Each level of biochar dose combined with each level of watering rates; mean ± SE, n=28. Different letters inside the graph denote significant differences between the treatments followed by two factor ANOVA (Post hoc Tukey test, $P < 0.05$).

3.4.2. Water stress alleviation by biochar

Both dosages of biochar increased soil moisture content. Soil moisture percentage increased up to seven-fold upon 2% biochar addition for both highest watering (200 ml per day) and lowest watering rates (40 ml per day) (Fig.24). In addition, biochar amendment showed significant effects on biomass at all watering rates (Fig.25a), but slightly less so at the lowest water addition (40 ml per day and 80 ml per day), where only the 2% biochar dosage but not the 0.5% dosage showed significant increments on biomass production (Fig.25a). However, biochar addition (2% w: w) was less effective under water-stressed conditions (+67% biomass at 40 ml water per day) than in the presence of ample water (+311% at 140 ml water per day). Thus, our study revealed that biochar improved soil moisture retention (Fig.24) but that this probably was not the main mechanism for increased maize biomass (Fig.25a). Thus, Hypothesis III-1 that biochar alleviates water stress thereby increasing maize growth was falsified based on our experimental data. In this respect our data are similar to those of Wang et al. (2016) where biochar addition improved soil moisture but not crop growth.

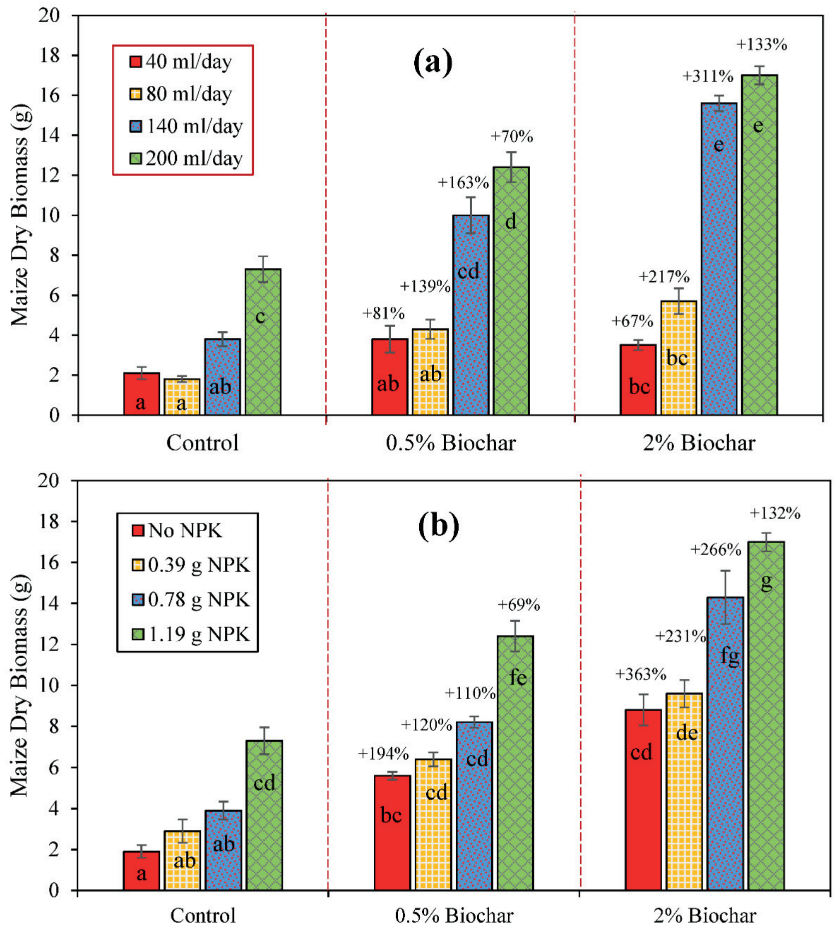


Fig 25. Dry weight of maize above ground biomass at harvest under water stress experiment (fig a) and nutrient stress experiment (fig b); mean ± SE, n=4. Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, $P < 0.05$). The percentage values above the bars denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar, at different watering (fig a) and NPK rates (fig b).

3.4.3. Nutrient stress alleviation by biochar

Biochar addition at 0.5% and 2% receiving low NPK (1/3rd NPK) increased maize biomass production by +120% and +231% respectively compared with control (Fig. 25b). A similar trend was observed for the combination of biochar and both the second and third NPK addition (Fig. 25b). Biochar addition at highest NPK rate also resulted in positive agronomic effects (+69% at 0.5% biochar and +132% at 2% biochar) but not as strong effects as those observed at low NPK addition at both 0.5% (+194%) and 2% biochar amendment (+363%) on maize biomass production (Fig. 25b). Thus, the most important effect of biochar in this soil was most likely nutrient stress alleviation. A recent study from (Jeffery et al., 2017) showed that biochar addition enhanced crop yield significantly in low fertility soils, highlighting the role of biochar in nutrient stress alleviation. However, in our study, biochar was still effective in the absence of nutrient stress (at highest NPK with an increase of +132%), probably due to other improved soil physicochemical (Table 7) or biological parameters (not explored in this study).

In this study, improved soil available K and P was probably the main nutrient factors (Table 5) responsible for increased biomass production. A significant positive relationship between maize biomass vs. K supply rates *(R^2=0.51, P<0.001)* and between maize biomass vs. P-AL *(R^2=0.61, P<0.001)* were observed (Fig.26). Other soil parameters such as NO_3^-, Mg^{2+}, Ca/Al ratio and Al^{3+} did not show significant correlation with maize biomass production in this soil. Positive relationship observed between maize biomass production and available K, combined with previous observations that K is the main nutrient added by the addition of biochar (Martinsen et al., 2014), indicating that the K addition via biochar contributed to the alleviation of nutrient stress by biochar. A recent study by Gautam et al. (2017) reported increased K^+ availability upon biochar addition (5 t ha^{-1}) in silty loam Nepalese soil. A similar positive trend was observed between maize biomass production and P-AL, probably due to increased P-AL, where biochar addition increased P-AL from 6 mg kg^{-1} up to a level of 80 mg kg^{-1} (Table 7) within the range of 50 -70 mg kg^{-1} required for optimal crop growth (Krogstad et al., 2008). Increased P-AL in P-poor soils was reported upon biochar addition, resulting in crop production improvements (Asai et al., 2009).

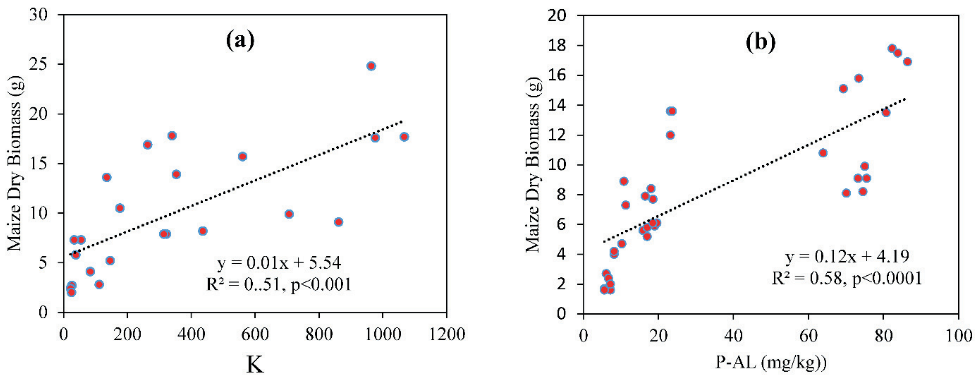


Fig 26. Relationship between maize biomass vs K (Fig a) and maize biomass vs. P-AL (Fig b) under nutrient stress experiment.

3.4.4. Acid stress alleviation by biochar

Both biochar and lime addition showed significant effects (*$P<0.001$*) on soil pH (Fig.27a). However, liming did not show significant effects (*$P>0.05$*) on maize biomass production (Fig.27b). Thus, soil acidity (pH of 4.5 in $CaCl_2$ and 5.1 in water and reasonably low exchangeable Al^{3+} of 1.6 $cmol_c\ kg^{-1}$) was not a limiting factor for crop production in this soil. In accordance with this, (Schmidt et al., 2015) reported no correlation between soil pH and crop yield explored under field trials (8 different sites) in silty loam Nepalese soil. Biochar addition was the only main factor increasing maize biomass production with respect to different liming rates in this experiment (Fig.27b).

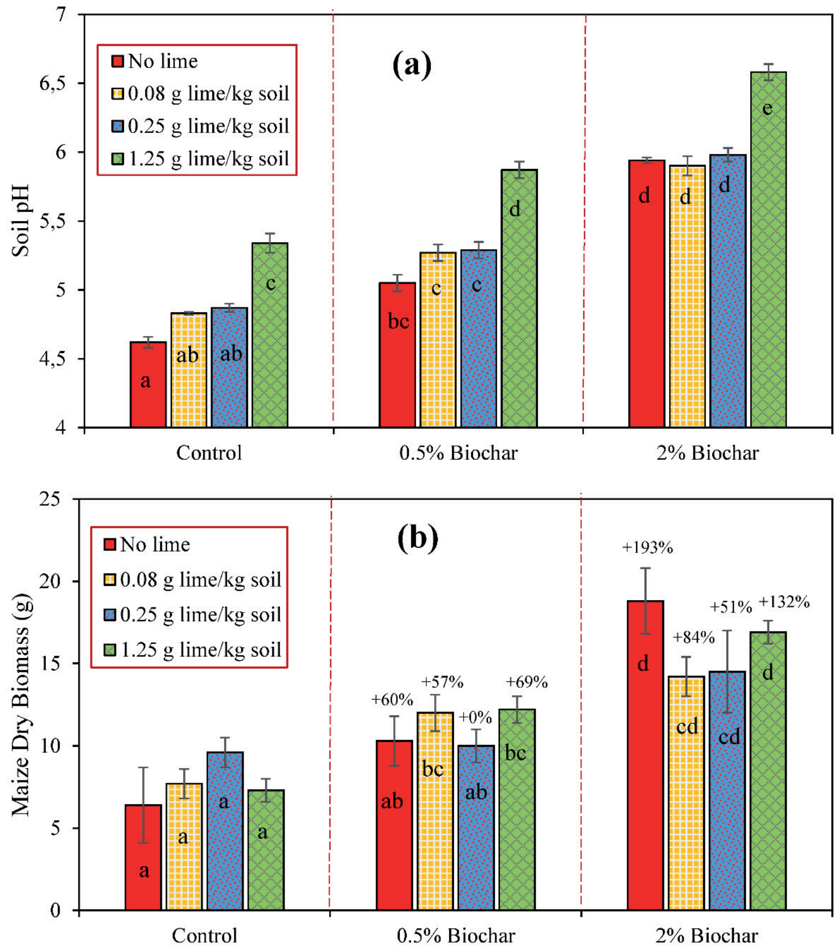


Fig 27. Effect of the combination of biochar dosages and liming rates on average soil pH (fig a; mean ± SE, n=11 and maize biomass production (fig b; mean ± SE, n=4). Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, $P < 0.05$). The percentage values above the bars (fig b) denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar.

In conclusion, hypotheses III-1 and III-3 were falsified with respect to water stress and acid stress respectively, whereas hypothesis III-2 (alleviation of nutrient stress) was supported by the experimental data.

3.5 Paper IV. Effect of biochar-compost mixtures on soil available nutrients and crop production

3.5.1 Composting conditions

The average moisture content was 5-15% higher for biochar-amended composts than for non-biochar composting piles for all three composting systems throughout the composting period (Fig.28a), which was mainly due to increased water holding capacity caused by biochar (Kammann et al., 2016; paper III of this thesis). Recorded temperatures were in the range of the mesophilic phase (below 40^0C) but a thermophilic phase (above 40^0C) was not reached, neither for compost nor for biochar co-compost of conventional and aerobic composting piles (Fig.28b). Similar to moisture content, average Eh was around 50mV higher for biochar-amended composts than for non-biochar composting ones (Fig.28c), possibly due to higher porosity of biochar that maintain the higher oxygen level for longer periods (Kammann et al., 2016). However, the measured values of Eh were slightly lower (below 500mV) (Fig.28c) than is normally expected following biochar addition (Eh > 500 mV), but were still in the range required for good soil quality (Husson, 2013).

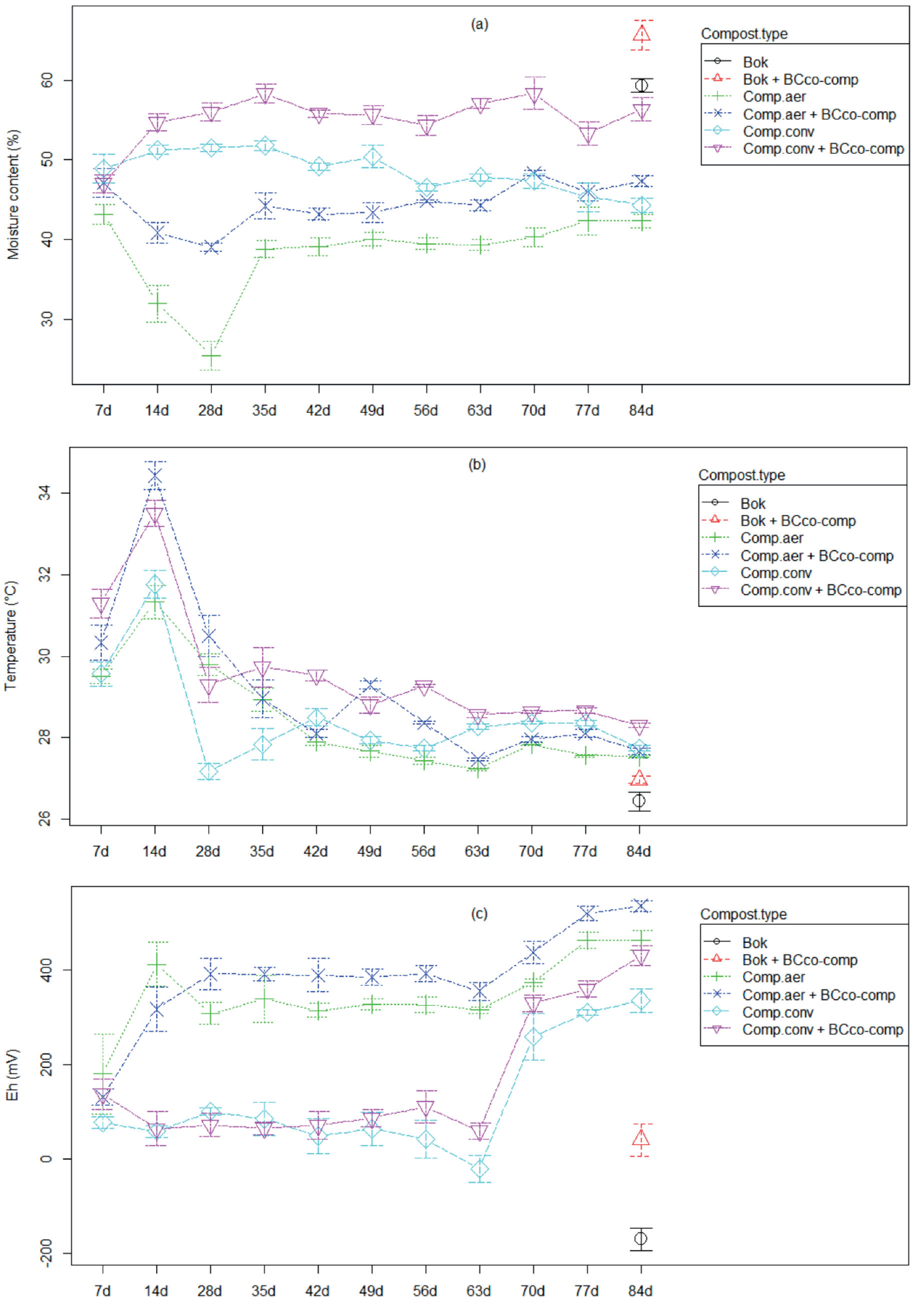


Fig 28. Average moisture content, temperature and Eh of different composting piles (y-axis) measured at every 7 day until compost harvest (x-axis), n=3; *Bok (bokashi fermentation), Bok + BCco-comp (bokashi co-composted biochar), Comp.aer (aerobic compost), Comp.aer + BCco-comp (aerobic*

co-composted biochar), Comp.conv (Conventional compost) and Comp.conv + BCco-comp (Conventional co-composted biochar)

Aerobic co-composted biochar (Comp.aer-BC) had highest pH (7.9 ± 0.1) and bokashi fermentation (Bok) showed lowest pH (pH 4.89 ± 0.04) (Fig.29). However, bokashi in the presence of biochar (Bok-BC) was not acidic (pH 7.20± 0.02) (Fig.29). Previous work showed that lactic acid fermentation also occurred at neutral pH (Probst et al., 2015).

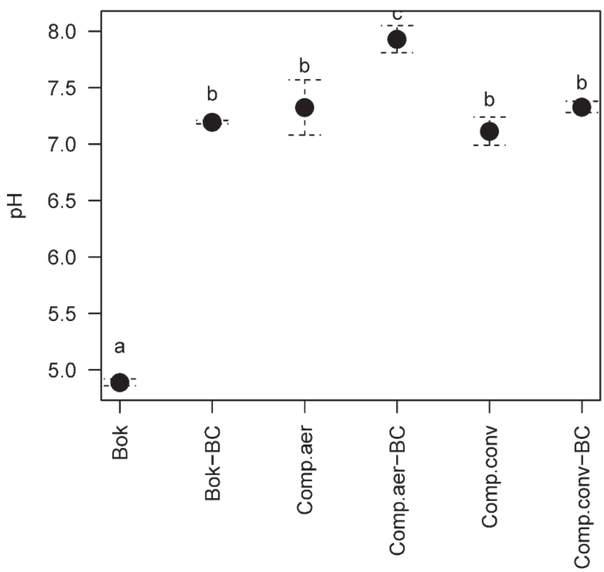


Fig 29. Average pH of composting piles, n=2 measured at day 40 and day 80 of composting process.

3.5.2. Soil physicochemical properties and available nutrients

Soil available P (P-AL) was significantly higher for both co-composted and post mixed biochar-compost mixtures (60 t ha^{-1}) from all three composting methods (44 to 105 mg kg^{-1}) when compared with NPK and NPK + BC treatments (34 and 38 mg kg^{-1} respectively) (Table 8). Much higher P-AL was observed for bokashi co-composted BC (105 mg kg^{-1}) than for all other organic amendments with and without biochar (ranging from 32 to 55 mg kg^{-1}). Similarly, soil moisture content, CEC and other exchangeable base cations (K^+, Ca^{2+}, Mg^{2+}) were observed highest for bokashi co-composted BC (Table 8). Co-composted biochar from aerobic composting also had higher soil P-AL and K^+ contents

(Table 8) compared with aerobic compost without biochar and NPK treatments. However, the availability of nutrients in soil with aerobic and conventional compost in the presence of biochar were far less than those observed for bokashi fermented biochar amendments. This was probably due to lacto bacilli amended in bokashi fermentation that enhance microbial organic degradation which increases nutrient availability in the soil system (Boechat et al., 2013). Beneficial effects of co-composted biochar on soil physicochemical properties and available nutrients (P-AL, K^+, NO_3^-) have previously been reported by Agegnehu et al. (2016). Increased nutrient retention was due to the formation of organic coatings in co-composted biochar, which entrap or adsorb dissolved nutrients in the system (Hagemann et al., 2017; Joseph et al., 2017). In this study, our data supported hypothesis IV-1 that biochar-compost formulations could enhance soil available nutrients (mainly P and K) for aerobic and bokashi co-compost but not with conventional co-composting.

Table 8. Effect of organic amendments (compost and co-compost) mixed with and without biochar (applied at 60 t ha^{-1}) on soil physicochemical properties. Different letters within each column denotes significant differences between treatments on soil properties following one-way ANOVA (*REG-WQ test, P < 0.05*)

Treatments	Moisture	K^+	Ca^{2+}	Mg^{2+}	CEC	P-AL
	%		cmolc/kg			mg/kg
NPK	8 ± 1a	0.7 ± 0.04c	4.0 ± 0.1a	1.2 ± 0.01a	7.8 ± 0.2a	34.8 ± 4.7ab
NPK+BC	11 ± 1b	1.0 ± 0.03e	4.5 ± 0.2b	1.2 ± 0.04a	7.8 ± 0.2a	38.3 ± 2.3ab
Conventional compost	11 ± 1b	0.7 ± 0.01c	5.7 ± 0.2c	1.8 ± 0.04b	8.4 ± 0.2b	43.0 ± 2.9bc
Conventional post-mixed BC	11 ± 1b	0.9 ± 0.08de	5.6 ± 0.2c	1.8 ± 0.11b	9.0 ± 0.7bc	44.0 ± 0.5c
Conventional co-composted BC	11 ± 1b	0.6 ± 0.02b	6.0 ± 0.2d	1.9 ± 0.08bc	8.6 ± 0.4bc	52.7 ± 7.0cde
Aerobic compost	11 ± 2b	0.5 ± 0.03a	6.2 ± 0.2d	2.0 ± 0.04c	9.3 ± 0.3c	32.1 ± 1.4a
Aerobic post-mixed BC	13 ± 2b	1.0 ± 0.06de	6.3 ± 0.0d	2.0 ± 0.00c	10.3 ± 0.1d	49.0 ± 2.2d
Aerobic co-composted BC	12 ± 2b	0.9 ± 0.02d	6.2 ± 0.5cd	2.0 ± 0.12c	10.5 ± 0.4d	55.1 ± 2.1e
Bokashi fermentation	11 ± 1b	0.9 ± 0.08de	4.9 ± 0.2b	1.6 ± 0.08b	9.8 ± 0.6cd	38.4 ± 1.4a
Bokashi post-mixed BC	16 ± 2c	1.3 ± 0.03f	6.1 ± 0.2cd	2.1 ± 0.04c	10.2 ± 0.3d	57.7 ± 2.3e
Bokashi co-composted BC	17 ± 2c	1.7 ± 0.12g	7.3 ± 0.6e	2.5 ± 0.11d	12.0 ± 0.9e	105.1 ± 2.8f

3.5.3. Biomass production

Co-composted bokashi (60 t ha^{-1}) significantly increased biomass production per pot by 243%, 204% and 149% compared with NPK, NPK+BC and bokashi without biochar respectively (Fig.30). Bokashi post-mixed BC also showed increased biomass production compared with NPK and NPK+BC, but less pronouncedly so (+132 % and +106%, respectively; Fig.30). In contrast, compost and BC-compost produced from conventional and aerobic systems did not reveal significant differences in effect on biomass production from NPK (control) and NPK+BC (Fig.30). In accordance with this, (Andreev et al.,

2016) reported significantly higher maize height upon bokashi fermented-biochar mixtures compared to a control, mineral fertilizers and other organic amendments (stored faeces, stored cattle urine and stored urine). Thus, hypothesis IV-2 that maize biomass growth would be increased as a result of increased soil nutrient availability was supported by the experimental data only with regard to bokashi-biochar mixtures especially co-composted biochar-bokashi formulations but not with conventional and aerobic biochar-compost formulations.

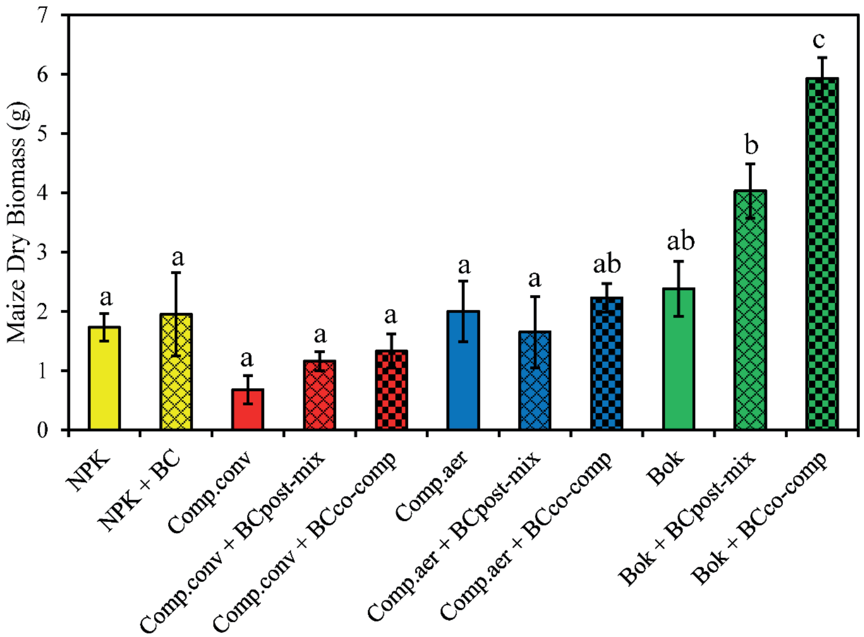


Fig 30. Effect of various organic and inorganic amendments in the presence and absence of biochar applied at the rate of 60 t ha^{-1} composts on maize biomass production (mean ± SE, n=4). Different letters above a bar of each treatment represents significant differences between various treatments following one way-ANOVA (post hoc-REG-WQ test, P < 0.05).

Among various soil factors explored as a function of organic and inorganic amendments (60 t ha^{-1}) on maize biomass production, soil P-AL (R^2= *0.55*) and exchangeable base cations such as K^+ (R^2= *0.64*), Ca^{2+} (R^2= *0.35*) and Mg^{2+} (R^2= *0.36*) showed significant positive relationships (*P<0.001*) with maize biomass production (Fig.31). Other soil factors such as soil NO_3^-, NH_4^+, pH and Eh did not show significant positive correlation with maize biomass production in this soil. Soil moisture content was not included to explore the relationship with maize biomass, as the measured moisture content (Table 8) was relatively low for all the treatments including bokashi-biochar mixtures (ranging from 8 to 17 % vol.), which was not considered the potential soil factor for improved crop growth. Similar to paper III, increased soil nutrient availability (mainly P and K) probably was the main soil factor responsible for increased biomass production in this soil.

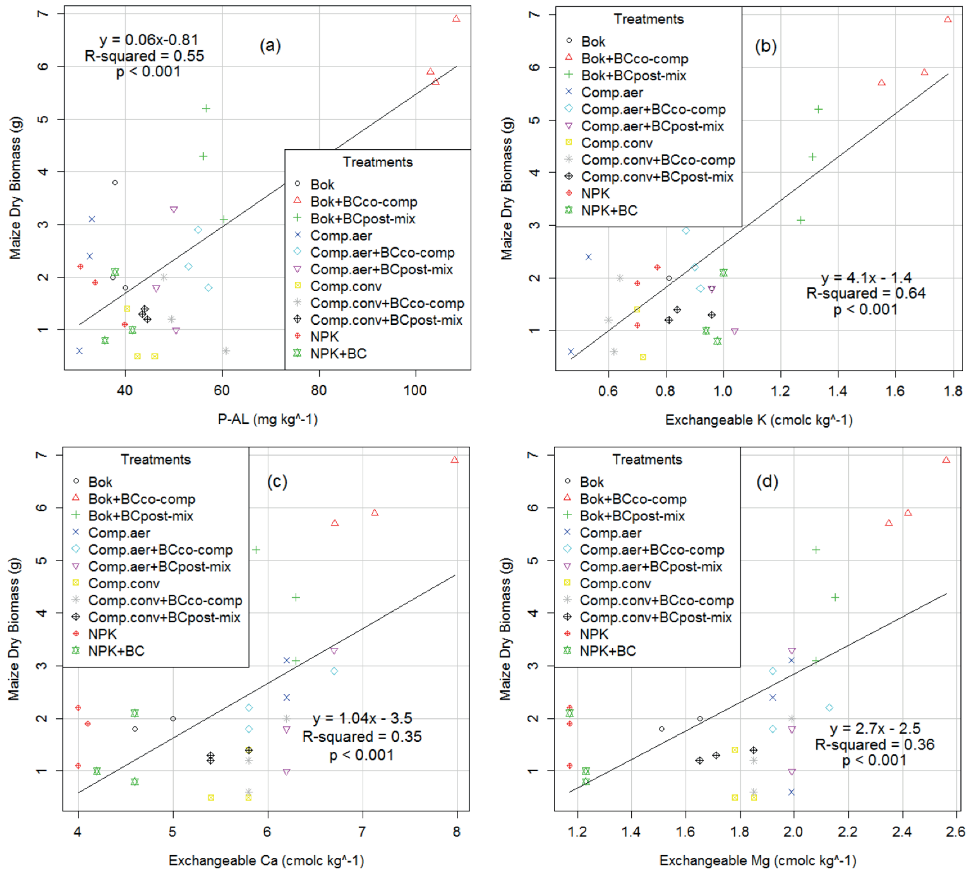


Fig 31. Relationship between P-AL and exchangeable base cations (K^+, Ca^{2+} and Mg^{2+}) with maize biomass for various organic and inorganic treatments applied at 60 t ha^{-1} of composts.

Optimal maize growth requires P-AL to be in the range of 50-80 mg kg^{-1} (Krogstad et al., 2008). Most of the organic amendment (including co-composted biochar from aerobic and conventional compost) and inorganic amendments used in this work had soil P-AL < 55 mg kg^{-1}, with the exception of co-composted biochar bokashi (> 70 mg kg^{-1}), providing a possible explanation for the superior effects on crop growth, similar to that as reported by Asai et al. (2009), where rice yield increased with higher amount of available P. Indeed, P deficiency symptoms were observed for many of the treatments including bokashi without biochar. No P deficiency symptoms were observed for bokashi-biochar formulations. Furthermore, co-composted bokashi also improved soil CEC mainly through increased exchangeable base cations such as K^+, Ca^{2+} and Mg^{2+} (Table 8), which all contributed to the beneficial effect observed for biomass production (Fig.31). There are many previous studies that have observed that the amendment of biochar results in higher amounts of exchangeable base cations esp. K^+ and concluded that these effects resulted in positive effects on crop production (Agegnehu et al., 2016; Martinsen et al., 2014; Yamato et al., 2006).

3.6 Paper V. Long-term agronomic effect of biochar

3.6.1. Agronomic effect of biochar over three year cropping

Biochar addition did not show significant effect ($p > 0.05$) on maize (Fig.32a) and mustard grain yield (Fig.32b) during the first year harvest (season 1 and 2). Maize grain yield (second year, third season harvest) increased by +50%, +47% and +93% at 15 t ha^{-1} BC, 25 t ha^{-1} BC and 40 t ha^{-1} BC addition respectively compared to control soil (Fig.32a). Similarly, at these biochar additions (15 t ha^{-1} BC, 25 t ha^{-1} BC and 40 t ha^{-1} BC), mustard grain yield (fourth season harvest) was increased by +96%, +128% and +134% respectively compared with control (Fig.32b). Similar significant trend as observed for second year harvest were observed on both maize and mustard crop yield for third year harvest. Both crop yields gradually increased with increasing biochar dosage over 10 t ha^{-1} (Fig.32). In accordance with this, Major et al. (2010) reported increased maize yield in repeated years (after first year) with the amendment of only 20 t ha^{-1} biochar but not for a dosage of 8 t ha^{-1} during four year field trials (maize-soybean rotation) in Colombian savanna Oxisol. Similarly, another study by Jones et al. (2012) reported significant effect of biochar on foliar N uptake and grass crop production only in second and third year harvest (not first year) at high biochar additions (25 t ha^{-1} and 50 t ha^{-1}) when applied in a Cambisol. The results indicate that biochar needs a certain level of aging in the soil in order to exert its positive yield effects. Haider et al. (2016) reported aged biochar to be more effective than fresh biochar in response to nutrient capture and delivery, which may lead to increased crop yield over time. As mentioned in paper IV, biochar after aging in compost can form organic coatings on biochar, increasing nutrient retention (Hagemann et al., 2017). In the experiment here, a similar phenomenon may have occurred over time in the presence of the repeatedly applied manure.

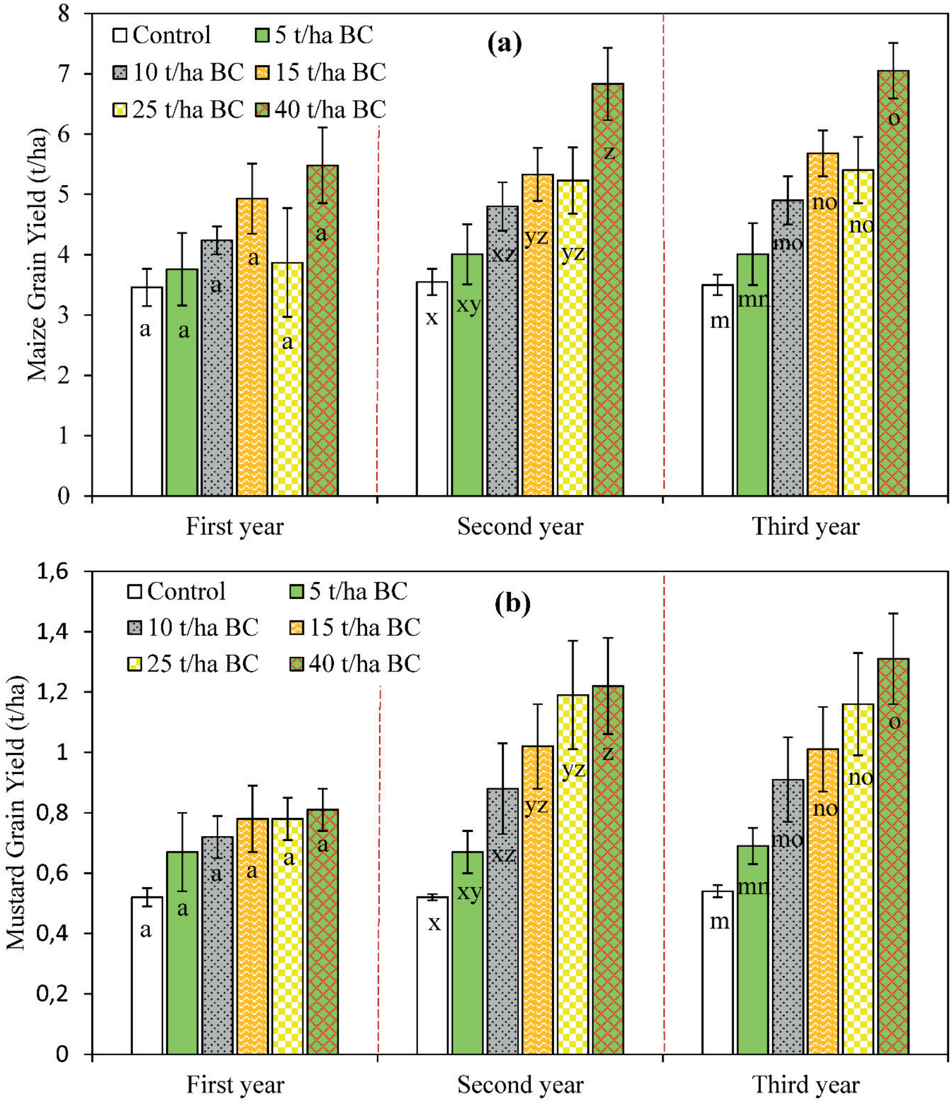


Fig 32. Effect of biochar addition on grain yield of maize (*fig a*) and mustard (*fig b*) over a period of three cropping years; mean ± SE, n = 4. Different letters inside a bar of each treatment represents significant differences between various treatments in a respective year following one factor ANOVA (post hoc-Tukey test, p = 0.05).

Biochar amendment (10 t ha^{-1} and 40 t ha^{-1}) significantly increased soil available P (P-AL), which showed significant positive relationship with crop grain yield ($R^2 = 0.95$, Fig.33a). In the present study, P-AL was increased from 12.5 to 65 mg kg^{-1} upon biochar addition (40 t ha^{-1}) (Fig.33a), similar to that observed in paper III and IV, which is in the range of 50-70 mg kg^{-1} required for proper growth and development of the plant (Krogstad et al., 2008). In addition, biochar amendment revealed positive effect (correlated) on soil chemical properties such as soil pH, OC %, CEC, BS and exchangeable K^+, which showed significant positive relationship with crop yield (Fig.33). This is corroborated with many previous field studies carried out in low fertile tropical soils where improved soil physiochemical properties (improved pH, CEC and base cations) has shown beneficial effect on crop yield (Cornelissen et al., 2013a; Martinsen et al., 2014; Yamato et al., 2006). Furthermore, biochar amendment increased soil moisture at field capacity and PAW by 5 % compared with control soil, similar to that as observed in paper III under greenhouse trial (Table 7). However, increased soil moisture retention was observed only at a high dosage of 40 t ha^{-1}, thus, moisture was not expected to be the main growth-limiting factor in this soil, in line with our greenhouse observations (Paper III). Thus, hypothesis V- 1 that biochar addition improves nutrient retention capacity, which in turn, increases crop yield for all three cropping cycles was accepted for second and third cropping year but falsified with respect to first year.

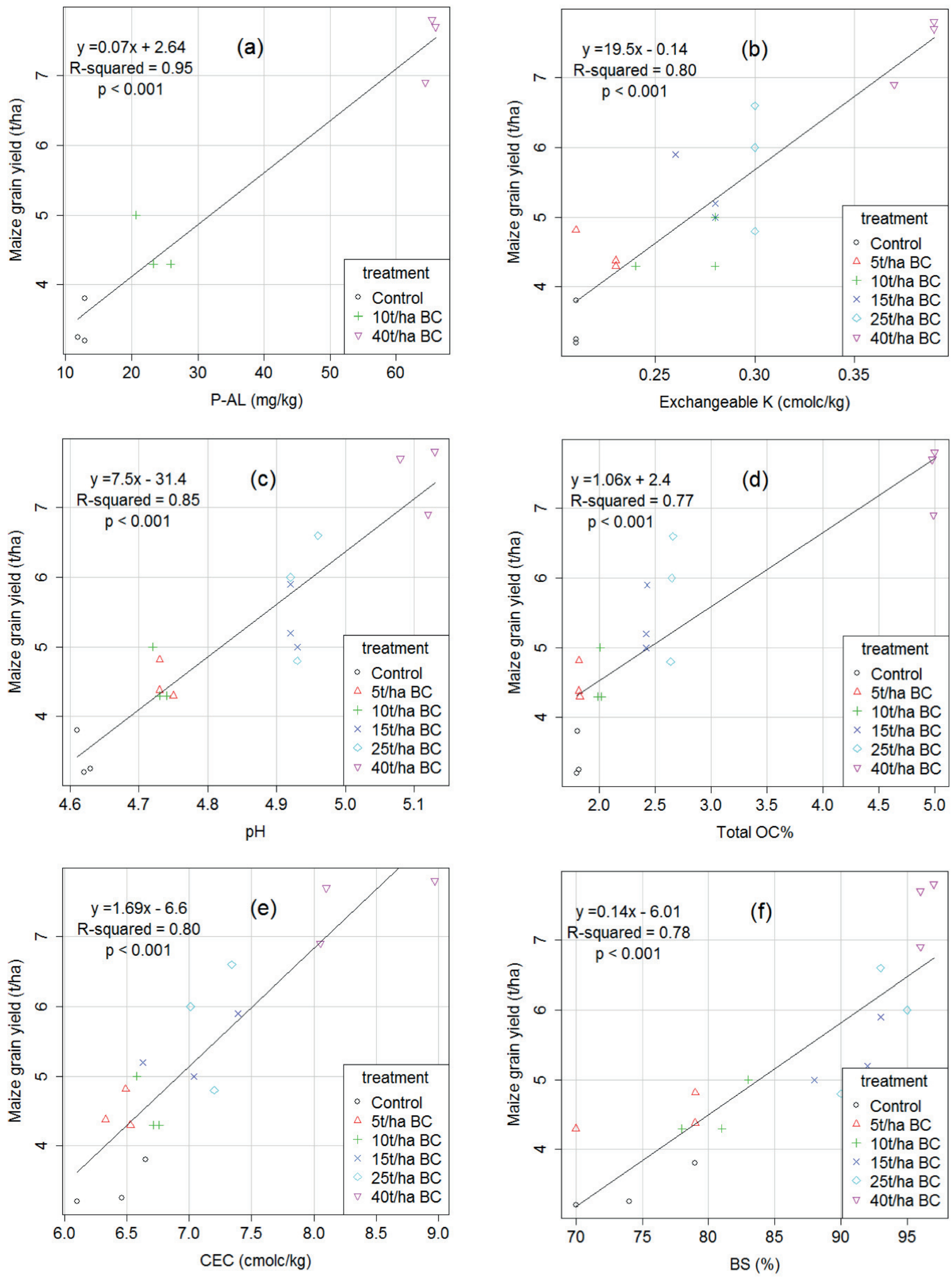


Fig 33. Relationship between soil parameters and maize grain yield (third year) as a function of biochar addition (n=18)

3.6.2. Effect of biochar addition on soil carbon

Biochar addition showed significant effect on total soil organic carbon (SOC) at all level of biochar addition (positively correlated with biochar dosages) except the lowest dosage (5 t ha^{-1} BC) (Fig.33d), indicating the stability of C in the biochar over 2.5 years (Kuzyakov et al., 2014; Wang et al., 2016). Total SOC showed significant positive relationship with crop yield ($R^2 = 0.77$, Fig.33d).

On the basis of the calibration that around 24% of condensed C is converted into benzenepentacarboxylic (B5CA) and benzenehexacarboxylic (B6CA) during the nitric acid oxidation (Bostick in revision), we calculate amounts of 91.6% condensed carbon in the pure biochar, 0.50 % in the non-amended soil, and 3.7 % in the soil amended with 40 t/ha biochar after 2.5 y (Table 9). Thus, 3.7 – 0.5 = 3.2% of the condensed C can be attributed to the added biochar, similar to the amount of biochar C originally added to the soil (4.99 – 1.81 = 3.18%, Table 9). This suggests that almost all condensed C in the biochar survived after five seasons (2.5 y) of aging under field conditions. The degree of aromatic condensation of the original carbon in the sample can be represented by the ratio of B5CA/BC6A (Schneider et al., 2010) as B5CA are formed from less condensed components than B6CA compounds . Thus, the 0.53 B5CA/B6CA ratio of biochar in the aged soil indicates it was less condensed, and perhaps more oxidized, than the fresh biochar with a B5CA/B6CA ratio of 0.35 (Table 9).

Table 9. BPCA composition of pristine biochar, aged biochar in the 40 t ha^{-1} plots (after 5 seasons) and the control soil.

Treatments	Total OC%	B5CA[1]	B6CA[1]	Pyrogenic C[2]	B5CA/B6CA
		mg BPCA per g soil		%	
Fresh biochar	70	57.3	163.5	91.6	0.35
Control soil	1.6	0.53 ± 0.03	0.68 ± 0.06	0.5	0.78
40 t ha^{-1} aged biochar	4.99	3.1 ± 0.3	5.8 ± 0.4	3.7	0.53

[1] Benzenepentacarboxylic (B5CA) and benzenehexacarboxylic (B6CA) acids

[2] (B5CA + B6CA)*4.1/10

3.6.3. Cost-benefit analysis

Gross margin per ha cropped land was observed highest (4500 US$) for 15 t ha^{-1} biochar addition, when calculated based on the medium social cost of CO_2 price (42 US$ per ton) (Fig.34c), taking into account the CH_4 emission cost during biochar production and income/benefit when burying it in soil (C sequestration) in the respective biochar addition plot. Without a carbon price, gross margin still peaked at a biochar dosage of 15 t ha^{-1}, but at a lower value of around 3481 US$ (Fig.34a), and showing a sharper decrease with increasing biochar dosage above 15 t ha^{-1} where the increased crop yield was not worth the investment of adding such high amounts of biochar. Currently there is no possibility for payment of C credits to farmers and this will provide small incentive for biochar use from the farmer's perspective as the difference in gross margin between no biochar amendment (3163 US$ over 3 y) and optimal biochar amendment rate (15 t/ha; 3481 US$ over 3 y) was only 10%. At a voluntary market C price of 6$ per ton CO_2 (Fig.34b) as well as at a medium social cost of CO_2 price of 42 US$ per ton (Fig.34c), gross margin also peaked at 15 t ha^{-1} biochar with the clearest incentive for making biochar at the 42US$ CO_2 price (gross margin for 15 t ha^{-1} biochar 4500 US$ ha^{-1} over 3 years, and for no biochar 3163 US$ ha^{-1}; a difference of 42%). At a high social cost CO_2 price (147$ per ton CO_2), gross margin continued to increase with biochar dosage, as theoretical income from such highly priced potential carbon credits would exceed that from crop yields (Fig.34d). This is one of the first studies taking into account both the climate cost of methane emissions and the health cost of CO and aerosol emissions during biochar production. However, these are not costs that are directly felt by the farmer making the biochar. Thus, the direct farmer incentive to make biochar is actually higher than represented in the graphs in Fig. 34 for those cases where C price is higher than zero. Based on the significant effect of biochar applied at 15 t ha^{-1} on maize crop (Fig.32a) and mustard crop (Fig.32b) in a subsequent year along with higher gross margin, this study suggests the optimal biochar dosage under local farmers practices is 15 t ha^{-1}. Thus, hypothesis V-2 that biochar can improve farming economics for small-scale farming in Nepal is supported upon application of 15t ha^{-1} biochar addition.

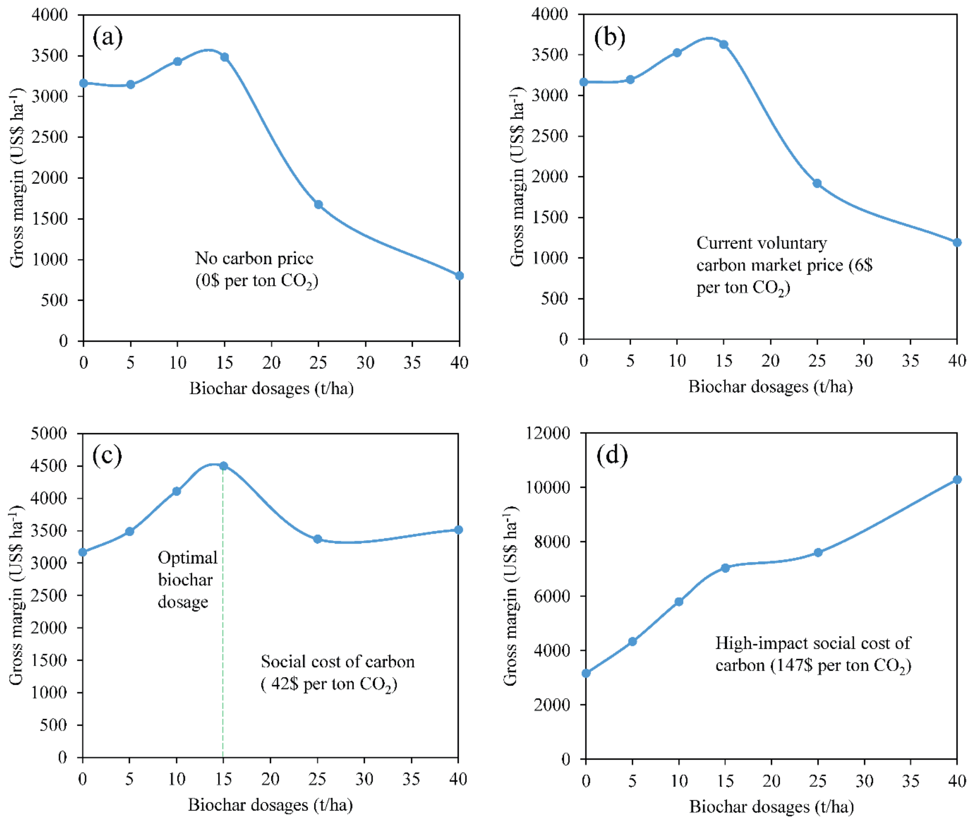


Fig 34. Gross margin of single biochar application at different levels, with varying carbon prices; a) no carbon price, b) voluntary market price (6$ per ton CO_2), c) medium social cost of C price (42$ per ton CO_2) and d) high-impact social cost of C price (147$ per ton CO_2) under maize and mustard cropping system over a three-year period.

4. Conclusion and outlook

In conclusion, biochar production from Eupatorium feedstock using flame curtain kilns was found to result in good quality biochar with relatively low gas and particle emissions during the production process compared with other non-retort (traditional) and retort kilns. The resultant biochar showed significant positive effect (*p<0.001*) on maize biomass production, especially when biochar was hot nutrient enriched. With respect to agronomic effects of biochar, the amendments were found to increase soil moisture and nutrient retention capacity in a moderately acidic Nepalese soil when explored under both field and controlled greenhouse conditions. Biochar addition significantly improved soil physicochemical properties such plant available water, pH, OC%, CEC, exchangeable base cations and soil nutrient availability (available P and K). The main working mechanism of the biochar for increased maize biomass production was probably increased nutrient availability (P and K). Under controlled greenhouse conditions, biochar addition was found to alleviate nutrient stress conditions thereby increasing crop production. Biochar did not alleviate water stress very much in this soil and lime addition did not show positive effect on crop growth, illustrating that moisture and pH were probably not the main growth-limiting factors in this soil. However, indirect pH effects on maize biomass were likely as there was a positive correlation between soil pH and available P ($R^2 = 0.75$, $P<0.01$). Maize is more sensitive to drought and nutrient conditions and relatively tolerant to low pH compared to other crops, thus, the results found for maize plant might not be fully representative for other plants. Repetition of the experimental design is recommended for various soils with various limiting factors for crop growth, as well as for various biochar and crop type. Based on BPCA analysis, the carbon sequestration benefit was clearly illustrated, with almost complete C stability, and almost no weathering of the biochar after 2.5 year of aging in this soil. We thus observed that biochar can contribute to climate change mitigation.

In this study, co-composted biochar produced from bokashi fermentation (lacto bacilli fermentation) was found to have strongly significant effects on soil physiochemical properties, available nutrients and crop growth. Superior crop growth of co-composted bokashi-biochar among other organic and inorganic amendments could possibly be explained by higher soil available nutrients, mainly P-AL and K^+ with minor contributions Ca^{2+} and Mg^{2+}. This is very relevant news for smallholder farmers, as it means that optimizing their nutrient management with locally available materials (biochar, manure, greenwaste) can actually lead to better harvests than the use of expensive, imported mineral fertilizers.

However, bokashi-biochar co-composting formulations was found effective at high compost addition rate (60 t ha^{-1}), but not at usual compost dosages of 20 t ha^{-1}. The high 60 t ha^{-1} dosage was used in order to gain a better understanding of the processes operating. Thus, more research is needed to find out whether the positive effect of adding bokashi-biochar formulations encompasses many soil types, or whether the effect was specific for the presently studied oxidized Inceptisol, where a high dosage was needed to improve the crop growth. The improved crop growth for bokashi fermentation in the presence of biochar was probably partly explained by increased nutrient availability (most notably P). Thus, based on our greenhouse experiment (paper III and IV) and field trial (paper V) observations, we can conclude that plant available P was one of the most important maize growth limiting factors in our soil, and that the P limitation could be significantly alleviated by biochar amendment.

In Nepal, *Eupatorium adenophorum*, an invasive shrub regenerated naturally in forest, farm upland and riverbanks could be effectively utilized without any financial cost to produce a biochar by farmers themselves at local conditions. One ton of biochar can be produced from around five ton of dry Eupatorium (20% conversion efficiency). Biochar (1 ton) could be produced at the financial cost of around US$ 144 using soil pit flame curtain, comprising the cost of labor (for feedstock collection and kiln operation), packaging, storing and transportation. However, this cost may vary based on the accessibility of feedstock and agricultural land where biochar would be applied. Other indirect costs included to produce one-ton biochar were the health cost of CO and aerosol emissions (13 US$) and environmental cost of methane emissions (11 US$, taking medium social cost of carbon, a cost hardly included in previous cost-benefit analyses of biochar).

Biochar addition under three year agronomic trial with maize and mustard farming was found economically viable for all dosages of biochar addition. Among various biochar dosages, the optimal amount was found to be 15 t ha^{-1} based on agronomical (crop yield), economic (cost benefit analysis) and environmental (C sequestration) considerations. For a zero C price regime (i.e., without payment for C sequestration, the current situation), gross margin was improved by around 10%, and drastically reduced for biochar rate exceeding 15 t ha^{-1} (25 t $ha^{-1}$and 40 t ha^{-1}), thus, the observed increased yield was not worth the investment of adding such high amounts of biochar. Taking a medium social carbon price (42 US$ per ton), a farmer could fetch a gross margin of around 4500 US$ per ha over 3 years (1500 US$ per ha per year), which would be an improvement of 42% compared to that from no biochar amendment (3163 US$ per ha over 3 years i.e. 1054 US$ per ha per year). The average household landholding size of the Nepal mid-hills is 0.7 ha (CBS, 2001/2002) and, thus, biochar application (15 t ha^{-1}) could increase the average margin per household by 3150 US$ over 3 years (1050 US$ per year) compared to control (2214 US$ over 3 years i.e. 738 US$ per ha per year). Increased margin of 42% through biochar amendment would significantly improve the socio-economic status of poor farmers in Nepal where 25% of rural household are still living below the poverty line (average household income < 1000US$ per year, NLSS 2011).

The main highlights of the thesis can be summarized as follows;

- Making biochar with the simple, free and low-emission flame curtain kiln technology can turn the pest "forest killer" into a valuable resource and contribute to climate change mitigation;
- Biochar increases crop harvest, mainly by improved plant nutrition;
- Biochar co-fermented with manure and greenwaste provides optimal nutrient management based on locally available materials;
- Biochar can increase the gross margin of smallholder farmers by 10-40%.

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Paper I.

Emissions and char quality of flame-curtain "kon-tiki" kilns for farmer-scale charcoal/biochar production

Gerard Cornelissen, Hans Peter Schmidt, Naba Raj Pandit, Paul Taylor, Bishnu Hari Pandit, Magnus Sparrevik

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RESEARCH ARTICLE

Emissions and Char Quality of Flame-Curtain "Kon Tiki" Kilns for Farmer-Scale Charcoal/ Biochar Production

Gerard Cornelissen[1,2]*, Naba Raj Pandit[2,3], Paul Taylor[4], Bishnu Hari Pandit[3], Magnus Sparrevik[5], Hans Peter Schmidt[4]*

1 Norwegian Geotechnical Institute (NGI), Oslo, Norway, **2** Institute for Environmental Sciences (IMV), University of Life Sciences (NMBU), Ås, Norway, **3** Nepal Agroforestry Foundation (NAF), Kathmandu, Nepal, **4** Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, Arbaz, Switzerland, **5** Department of Industrial Economics and Technology Management, Norwegian University of Technology, Trondheim, Norway

* gco@ngi.no (GC); schmidt@ithaka-institut.org (HPS)

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Abstract

Flame Curtain Biochar Kilns

Pyrolysis of organic waste or woody materials yields charcoal, a stable carbonaceous product that can be used for cooking or mixed into soil, in the latter case often termed "biochar". Traditional kiln technologies for charcoal production are slow and without treatment of the pyrolysis gases, resulting in emissions of gases (mainly methane and carbon monoxide) and aerosols that are both toxic and contribute to greenhouse gas emissions. In retort kilns pyrolysis gases are led back to a combustion chamber. This can reduce emissions substantially, but is costly and consumes a considerable amount of valuable ignition material such as wood during start-up. To overcome these problems, a novel type of technology, the Kon-Tiki flame curtain pyrolysis, is proposed. This technology combines the simplicity of the traditional kiln with the combustion of pyrolysis gases in the flame curtain (similar to retort kilns), also avoiding use of external fuel for start-up.

Biochar Characteristics

A field study in Nepal using various feedstocks showed char yields of 22 ± 5% on a dry weight basis and 40 ± 11% on a C basis. Biochars with high C contents (76 ± 9%; n = 57), average surface areas (11 to 215 m^2 g^{-1}), low EPA16—PAHs (2.3 to 6.6 mg kg^{-1}) and high CECs (43 to 217 $cmol_c$/kg)(average for all feedstocks, mainly woody shrubs) were obtained, in compliance with the European Biochar Certificate (EBC).

Gas Emission Factors

Mean emission factors for the flame curtain kilns were (g kg^{-1} biochar for all feedstocks); CO_2 = 4300 ± 1700, CO = 54 ± 35, non-methane volatile organic compounds (NMVOC) = 6 ± 3, CH_4 = 30 ± 60, aerosols (PM_{10}) = 11 ± 15, total products of incomplete combustion (PIC) = 100 ± 83 and NO_x = 0.4 ± 0.3. The flame curtain kilns emitted statistically

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significantly ($p<0.05$) lower amounts of CO, PIC and NO_x than retort and traditional kilns, and higher amounts of CO_2.

Implications

With benefits such as high quality biochar, low emission, no need for start-up fuel, fast pyrolysis time and, importantly, easy and cheap construction and operation the flame curtain technology represent a promising possibility for sustainable rural biochar production.

Introduction

Biochar, a heterogeneous substance rich in aromatic carbon and minerals, is produced by pyrolysis of sustainably obtained biomass under controlled conditions. Biochar has been suggested to be used as a soil amendment to improve crop productivity especially in weathered and eroded tropical soils [1–3]. While the production of biochar in modern industrial devices can be a highly controlled process with low noxious emissions and resulting in certifiable high quality biochar [4, 5], the technology has elevated costs of investment (> US$ 500,000) and maintenance leading to current market prices in the range of US$ 600–900 per ton of biochar [6–8]. In developing countries where most of the weathered tropical soils are found, biochar is not an option at these costs.

Many charcoal-containing Terra Preta soils in e.g. the Amazonas region, Germany, Australia, China and Scandinavia [9] prove, however, that ancient people must have known how to produce large quantities of biochar without the help of modern steel-based technology.

As charcoal was necessary to reach the temperature for iron ore melting, the history of civilization has been linked to charcoal production since the beginning of the Iron Age. For more than 3000 years most charcoal was and still is in many developing countries produced with inefficient and polluting methods since syngases with significant caloric value are released into the atmosphere. These include methane, carbon monoxide (CO) and aerosols (smoke; $PM_{2.5}$ or PM_{10}), nitrogen oxide and dioxide (NO and NO_2, together NO_x), as well as non-methane volatile organic matter (NMVOC), in addition to hydrogen [10]. Many of these gases are deleterious to human health, and/or they exacerbate anthropogenic radiative forcing. Cleaner but simple and accessible charcoal-making technologies are thus desirable.

Several traditional and low cost technologies to produce charcoal exist. They are either based on traditional methods practiced for centuries already or were adapted with more modern materials like mild steel to improve their efficiency. In most cases they are not used to produce biochar for agriculture but to produce charcoal for cooking or for export [11]. For tropical rural settings, the most important challenges have been to introduce a technology that is affordable and preferably free to farmers [12], as well as one that generates as low as possible gas and particle emissions.

The most important low-technology production methods for biochar include:

1. *Traditional earth mound or earth covered pit kilns* usually deliver good quality biochar though only high-value wood logs can be used as feedstock. The main environmental drawback is that toxic pyrolysis gazes are emitted unburned into the atmosphere generating significant gas emissions [10]. In addition yields are relatively low (10–20%) [10, 12] and the pyrolysis process is very slow, taking several days.

2. The development of the *Adam retort kiln and similar devices such as basic steel retort systems* introduced the partial afterburning of pyrolysis gazes. In these retort systems the feedstock wood can be mixed with dry biowaste materials like prunings, rice husks or maize cobs but a lot of valuable start-up wood is still needed [12, 13]. Such medium-scale improved retort technologies, where the pyrolytic gases are recirculated into the combustion chamber and combusted internally [14], produce around 75% lower deleterious gas emissions (mainly CO, CH_4, aerosols) and higher conversion efficiencies of 30–45% than traditional systems. Energy contained in the recirculated carbon- and hydrogen rich syngases is thus used to sustain the pyrolysis process so that less heat from the endothermic pyrolysis reactions is needed to sustain the process [12, 13]. Moreover, the recirculation of pyrolytic gases leads to enhanced secondary char formation which also increases yield [15, 16].

3. Household-scale cooking stoves, so-called *TLUDs (Top-Lit Up-Draft stoves)* [17] can generate biochar while using the energy produced for cooking. Advantages include that they burn cleanly avoiding negative health effects due to indoor air emissions [18], can use various waste biomasses as feedstock and are fuel-efficient. Pyrolytic gases are mostly combusted in the flame front, reducing emissions of CO, CH_4 and aerosols by around 75% [19, 20] compared to traditional cooking. Small-scale TLUDs may be applicable for horticulture or small kitchen gardens [21] but they generate too little biochar (0.5–1 kg per run for household devices and up to 10 kg for the bigger community stoves) to supply enough biochar for farming or selling as charcoal. In addition, the stove needs to be actively quenched after each cycle, which is impractical in daily use.

Thus the implementation of biochar into agricultural practice and the efficiency of the charcoal industry have been hindered by the absence of a low or zero-cost but clean charcoal-producing technology that would allow the on farm production of high-quality charcoal in sufficient amounts. A recent development has been the introduction of the *Kon-Tiki* flame curtain kiln, designed in 2014 in Switzerland and rapidly spreading since by open source technology transfer to farmers in more than 50 countries [22].

One run of a 2 m^3 flame curtain kiln with an upper diameter of 2.4 m produces 500 kg of biochar (dry matter basis) and close to 2 MWh of heat from shrubs, husks, straw, prunings and other organic farm waste in about three hours needing one worker to maintain and control the process. In contrast to medium-sized retort kilns, no startup wood is needed for flame curtain kilns. The cost per kiln varies with design, construction material and country but is within a range of €30 (soil pit shield) to €5000. The cheapest way is a mere conically shaped soil pit which would essentially be for free.

In this paper, the gas and particle emissions of various flame curtain kiln designs were investigated, as well as the quality of the resulting biochars. To this end, 17 runs were performed with different feedstock mixtures in six different flame curtain kiln types, at Matathirta, a suburb of Kathmandu, Nepal. The basic feedstock was *Eupatorium adenophorum*, a very frequently occurring invasive forest shrub species that local people call "ban mara" (i.e. forest killer) [23] which is around 1–2 m high with stems up to 2 cm thick. Eupatorium was either pyrolyzed alone or blended with mixed firewood or rice husk. Gas and particle emissions (CO_2, CH_4, CO, NMVOC, aerosols/PM_{10}, NO_x) were determined, as well as biochar characteristics (elemental composition, specific surface area, polycyclic aromatic hydrocarbon (PAH) content, cation exchange capacity (CEC)). Thus, this paper provides important information on the performance and sustainability of a new, rapidly spreading biochar and charcoal making technology at an early stage.

Materials and Methods

Principle of the flame curtain kiln

The principle of the flame curtain pyrolysis consists of pyrolyzing biomass layer by layer in a conically formed metal kiln or soil pit (S1 Fig). A fire is started in the kiln, and the burning embers spread to form a first layer on the bottom of the kiln. A thin layer of biomass is then added on top of the embers, heats quickly and starts outgassing. The rising pyrolysis gas is caught in the flames and reacts with combustion air entering the kiln from the top. When ash appears on the outside of the carbonizing biomass, the next layer of biomass is homogenously spread on top. Convective and radiant energy from the flames above and from the hot pyrolyzing layers below heat the fresh biomass layer, which starts to pyrolyze [24].

The biochar below the upper pyrolysis layer is shielded from oxygen access by the fire curtain itself. The combustion zone thus forms a flame curtain that protects the underlying biochar from oxidizing and cleanly burns all pyrolysis smoke and gases as they pass through this hot fire front. It is important to spread each new biomass layer at the right time and rate determined by monitoring the flame, smoke and ash formation. Too much feedstock will smother the flame (producing smoke and gas emissions), and too little feedstock will not maintain a full curtain of flame to protect the biochar from oxidizing (forming ash) and to completely combust the pyrolysis gases (avoiding smoke). The manual layering of biomass is repeated until the metal kiln or soil pit is filled. The pyrolysis process is then actively ended by quenching with water or a nutrient solution (e.g., urine, dissolved fertilizer) or, where water is not easily available, by snuffing with a layer of soil (see S1 Fig for an illustration of the quenching and snuffing process).

The temperature in the main pyrolysis zone just below the flame curtain is 680°C to 750°C [22, 24] and cools down slowly below the main pyrolysis zone when new feedstock layers are added to 150–450°C depending on the duration of batch before final quenching. When snuffed with soil, biochar temperature may be maintained at above 400°C for more than 24h depending how tight the snuffing layer and kiln are.

Kiln designs

Five different kiln designs (deep cone metal kiln, soil pit kiln, metal-shield soil pit kiln, all with a capacity of 60–130 kg feedstock per run, and small shallow octagonal kiln, shallow and deep pyramid kilns, all with a capacity of 15–25 kg feedstock per run) were tested with different feedstock and feedstock blending (wood, eupatorium shrubs, rice husks) producing between 120 to 800 l biochar per run. The essential difference between the kilns was the diameter, the outer angle and the material of the kiln (see Table 1 and S1 Fig).

Moisture content

Prior to the startup of each run, the feedstock for pyrolysis was weighed. Moisture in the feedstock was measured with a *Voltcraft FM-300* Wood Humidity Meter at 1% accuracy. The Eupatorium contained 25% moisture, whereas the firewood and the rice husk contained 15% moisture. The mass and volume of the biochar were measured directly after water quenching or soil quenching. Dry mass of biochar was analyzed by drying at 110°C until mass equilibrium [12].

Biochar characterization

Carbon content in feedstock (and char) was measured in triplicates on 100-mg samples that were combusted at 1030°C and analyzed in an element analyzer (Perkin-Elmer Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES)). Wood feedstock

Table 1. List over the experimental runs, feedstocks, masses, biochar yields (both as % of total mass and as % of C), biochar C, H, N contents, surface areas (SA), Cation Exchange Capacities (CEC) and PAH contents. CEC both for unwashed (including soluble ash, i.e., both exchangeable bases and soluble cations) and washed biochar (soluble ash removed, i.e., the "real" CEC), with the difference being the apparent CEC stemming from soluble cations in the ash ("CEC ash").

	Feedstock ratio			Quench	Biochar											
	Eupatorium	Wood	Rice husk		C	H	N	Mass Yield	C yield	CEC Unwashed	CEC Washed	CEC Ash	SA	Total PAH	PAH excl. NAP[d]	BaP
	%	%	%		%	%	%	%	%	$cmol_c$/kg	$cmol_c$/kg	$cmol_c$/kg	m^2/g	mg/kg	mg/kg	mg/kg
All-steel deep octagonal																
	100	0	0	Water	77.0 ± 0.8	n.d.	n.d.	19	36		121		84.9		3.7	0.016
	80	20	0	Water	78.7	2.1	0.80	17	31		97					
$BC_{E\text{-}wood}$ [c]	50	50	0	Water	80.5	1.89	0.6	18	32		60		149[c]	2.3[c]		
Steel-shielded soil pit																
	100	0	0	Soil	71.2 ± 2.4	n.d.	n.d.	25	44	121	55	66	35.4		1.9	0.013
	80	20	0	Soil	88.8 ± 0.3	n.d.	n.d.	32	66	82	48	33				
	50	50	0	Soil	83.6	2.7	0.54	31	58	50	43	7				
Conical soil pit																
$BC_{E\text{-}soil}$ [c]	100	0	0	Soil	71.7	1.41	0.66	18	31	95	68	27	111[c]	6.6[c]		
	80	20	0	Soil	85.3 ± 2.1	n.d.	n.d.	27	54	63	55	8	74.6		2.0	0.037
	50	50	0	Soil	80.4	2.1	0.59	25	44	80	56	24				
All-steel shallow pyramidal and octagonal kilns																
Pyr 45° [a]	100	0	0	Water	75.3 ± 2.3	1.3	1.04	21	39				215[c]	4.9[c]		
Pyr 45°	50	50	0	Water	74.1 ± 2.0	n.d.	n.d.	20	37		97					
Pyr 55°	100	0	0	Water	76.5 ± 0.2	2.0	0.72	17	32		101		72.9		4.2	0.020
Pyr 55°	100	0	0	Water	84.1	n.d.	n.d.	20	42		82					
Oct 55° [b]	50	0	50	Water	54.7 ± 1.6	2.2	0.68	25	34				10.8		4.5	0.058
Pyr 45°	50	0	50	Water	55.0	n.d.	n.d.	25	34		45					
$BC_{E\text{-}met}$ [c] Oct 55°	100	0	0	Water	72 ± 1.1	1.33	0.54	13	22		130					
Pyr 45° heat shield	100	0	0	Water	72.5 ± 1.8	n.d.	n.d.	27	49		217					

[a] Pyramidal-shaped, angle 45 degrees.

[b] octagonal-shaped, angle 60 degrees.

[c] The biochars $BC_{E\text{-}wood}$, $BC_{E\text{-}soil}$ and $BC_{E\text{-}met}$ were analyzed according to the EBC certificate;

[d] PAH content excluding naphthalene.

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was analyzed to contain 50.1% C, Eupatorium shrub 40.3% C, and in our parallel project in Tanzania rice husk was analyzed to contain 41.1% C, in accordance with literature values [25]. All biochars were characterized for cation exchange capacity by extraction with ammonium acetate at pH 7, both before and after washing with water for those samples where quenching was done with soil, and only after washing for the water-quenched samples [26]. Three biochars representing two different kiln types (soil pit kiln and metal cone kiln each 70°—1m50 diameter) and two feedstock (100% Eupatorium and 50:50 Eupatorium: hard wood) were analyzed by a EBC accredited laboratory following the EBC certification program and methods [4, 27]. Five example biochars were further analyzed for 15 individual PAHs by 36-h exhaustive toluene Soxhlet extraction according to published procedures [28, 29] and surface area by N_2 adsorption at 77 K.

Gas emission factors

The gases analyzed were CO_2, CH_4, non-methane volatile organic carbon (NMVOC), nitric oxides (NO_x) and aerosols (total suspended particles, TSP, derived from PM_{10}, for details see SI and [10]). Based on the measurements the value for total products of incomplete combustion (PIC) was given by summarizing the values for CO+NMVOC+CH_4 and TSP (from PM_{10}). A Microtector II 6460 was used to analyze carbon dioxide (CO_2) and methane (CH_4), both with a detection limit of 0.1% by infrared sensors and non-methane volatile organic components (NMVOC) with a detection limit of 0.1 ppm by photoionization detection (PID). The PID was calibrated using isobutene. Carbon monoxide (CO) and nitric oxide (NO) were analyzed with a Kigaz 300 flue gas analyzer by internal jacket type electrochemical sensors. Detection limits were 1 ppm for both sensors. For CO values above 8000 ppm the Kigaz instrument internally dilutes the gas stream to be able to measure concentrations up to 50 000 ppm. The instrument converts NO to generic nitric oxides (NO_x) by applying a conversion factor of 1.03, thus assuming that 97% of NOx consists of NO. Particles in the form of PM_{10} were analyzed with a Thermo Scientific pdr-1500 instrument by use of photometric detection of particles (detection limit 0.1 $\mu g/m^3$).

For conversion of concentration from mass units to molar ratios in the particle measurements, all particles were assumed to consist of elementary carbon. For subsequent conversion from TSP to total suspended particles (PM_{10}) a conversion factor of 1.4 was used, thus assuming around 70% content of PM_{10} in the samples [12]. All sensors except the particle analyzer were protected by a 0.45µm particle filter that was changed regularly during measurements. Readings were taken as composite samples from the chimneys of the kilns during the pyrolysis process. Between three and ten readings were taken during the process depending on the duration of the charring. For further details and limits of detection, see S1 Description. In order to calculate the emission factors of the kilns the carbon balance method was utilized [10, 12, 30]. In this method, only the emission ratios between the gases are measured without the need to register the absolute mass of gases emitted. Instead, this mass is calculated by performing a carbon balance between the biomass entering the process and the biochar produced. From ten to twenty single-point ratios, time-weighted average values were calculated. Net molar component-to-CO_2 emission ratios for the measured gases and TSP (from PM_{10}) for the flame curtain runs were 0.02 for CO, 0.02 for CH_4, 0.001 for NMVOC, 0.01 for TSP and 0.0001 for NO_x. These ratios were used to calculate the emission factors in g per kg biochar produced. Details of the calculation method can be found in ref. [12] and are presented again in S2 Description.

Statistics

A two sample t-test with nonsimilar variance using R was used to test for effects of kiln type on gas emission factors (CO_2, CO, VOC, CH_4, TSP, PIC and NO). The emission factors for the flame curtain kilns were compared to those of traditional kilns and retort kilns measured in different countries and for different feedstocks but with exactly the same instruments [12]. Differences with p-values < 0.05 were considered significant.

Results and Discussion

Biochar yields

Biochar yields were 22 ± 5% on a dry weight basis and 40 ± 11% on a C basis (Table 1). This is in the same order of magnitude as other high temperature (700°C) pyrolysis systems [31–34]. It is also in the same order of magnitude of traditional low-temperature kilns but lower than low temperature retort kilns (typically around 30–40% on a dry weight basis [12, 13]).

Yields were significantly higher for the soil-quenched kilns (26 ± 5%) than for the water-quenched kilns (20 ± 4% including the rice husk/eupatorium runs, 19 ± 5% excluding these) (Table 1), mainly because of the dissolution and wash off some of the ashes in the water-quenched kilns and probably also because of the inevitable mixing of the biochar with soil minerals from the kiln and snuffing layer. Biochar yields were rather variable (13 to 32%), probably due to variation in operation conditions (frequency of biomass addition) and meteorological conditions (wind, air moisture, temperature) but also reflect "real-world" conditions where biochar yields with this method can be expected to be equally variable. Further factors influencing the biochar yield in flame curtain kilns are water content, particle size and bulk density of the feedstock. The higher the water content of the feedstock, the more combustion energy is needed to evaporate the water and to heat the feedstock to pyrolysis temperatures above 300–400°C. This leads to longer exposure times of feedstock material to the reduced combustion air at the kiln surface, which causes more surface carbon to oxidize and results in higher ash content and lower biochar carbon yield. Equally, the duration of complete pyrolysis of the core of larger diameter wood pieces is much longer than for higher surface low diameter feedstocks like grain husks (rice husks) or shrub twigs (eupatorium). Such differences in pyrolysis duration explain higher carbon losses and thus lower yields of wood logs compared to twigs, straw or husks.

Char characteristics

C contents of the chars were 75.5 ± 9% (n = 37; Table 1), the lowest value being for the rice husk / Eupatorium 50/50 mixed feedstock runs (54–55%), due to the high inorganic (silica) content of the rice husk [35]. H contents of nine example biochars were 1.85 ± 0.5%, N contents were 0.69 ± 0.16% and C/N ratios were 118 ± 28. The three EBC tested biochars have molar $H:C_{org}$ ratios of 0.22 to 0.28 and molar $O:C_{org}$ ratios of 0.04 to 0.07 confirming the high aromaticity expected for biochars made at temperatures around 700°C [36]. Surface areas of most biochars were in the range of 100–200 m^2/g (Table 1) which is in agreement with other biochars produced with industrial technology at temperatures of 600° to 750°C [37].

Cation Exchange Capacities (CECs) of 15 biochars were 40–130 cmolc/kg, with one char even showing CEC above 200 cmolc/kg, which is on the high end of literature values for field-made biochars [26, 38, 39], indicating that the biochars probably have good nutrient-holding characteristics [26, 40]. For the soil-quenched chars, up to half of the "apparent" CEC for unwashed chars actually stemmed from soluble base cations in the ashes (Table 1).

Looking more closely at the three more completely characterized biochars (Table 2), the most apparent difference is the ash content being higher in both eupatorium biochars ($BC_{E\text{-}met}$: 21.9% and $BC_{E\text{-}soil}$: 19.9%) compared to the eupatorium-wood biochar ($BC_{E\text{-}wood}$: 10.2%). This can be explained by the higher mineral content of eupatorium shrubs compared to hard wood and is confirmed by the much higher silica (34,000/34,000 vs 5400 g kg^{-1}), iron (6,000/3,700 vs 950 g kg^{-1}) and potassium (28,000/36,000 vs 19,000) content of the pure eupatorium chars. The nutrient contents further differed slightly between the two eupatorium chars which can be explained by the fact that the metal cone biochar was water quenched and lost a higher portion of soluble minerals while the concentration of some less soluble minerals increased compared to the soil snuffed biochar. This is illustrated most clearly by the highly soluble Na which was 5.5 times lower in the water quenched $BC_{E\text{-}met}$ (520 mg/kg) than in the soil snuffed $BC_{E\text{-}soil}$ (2900 mg/kg). The higher mineral content of both pure eupatorium chars is probably also the reason for the higher pH (9.8 / 9.6) compared to the eupatorium-wood char (8.7) which had also been water quenched.

The heavy metal contents were all low compared to the EBC thresholds indicating clean biomass feedstock. Interestingly, the zinc content of the pure eupatorium chars was comparably

Table 2. Analyses of three biochars made in three different kilns and with two different feedstocks. Analyzed by an EBC accredited laboratory following the EBC biochar analytical methods [4, 27] and compared to the EBC thresholds for premium and basic biochar quality.

Biochar name Kiln		$BC_{E\text{-}met}$ 60°—1.1 m steel	$BC_{E\text{-}soil}$ 70°—1.5m soil pit	$BC_{E\text{-}wood}$ 70° 1.5 m steel	EBC—threshold	
Biomass		**Eupatorium**	**Eupatorium**	**Eupatorim—Wood (50:50)**	**premium**	**basic**
Density	kg m^{-3}	120	n.d.	n.d.		
Specific surface (BET)	m^{-2} g	215	149	111		
Ash 550°C	mass-%	21.9	19.9	10.2		
Hydrogen	mass-%	1.33	1.41	1.89		
Carbon	mass-%	72	71.7	80.5		
Nitrogen	mass-%	0.54	0.66	0.6		
Oxygen	mass-%	4.0	6.2	6.7		
Carbonate CO2	mass-%	2.24	1.3	1.81		
Organic carbon	mass-%	71.4	71.3	80.0	> 50	> 50
H/C org. (molar)		0.22	0.24	0.28	< 0.7	< 0.7
O/C (molar)		0.042	0.07	0.06	< 0.4	< 0.4
pH		9.8	9.6	8.7		
Electric conductivity	μS cm^{-1}	9090	n.d.	n.d.		
Salt content	g kg^{-1}	53.7	n.d.	n.d.		
Phosphorous	mg kg^{-1}	3700	4600	3800		
Magnesium	mg kg^{-1}	12000	4100	3800		
Calcium	mg kg^{-1}	17000	15000	26000		
Potassium	mg kg^{-1}	28000	36000	19000		
Sodium	mg kg^{-1}	520	2900	860		
Iron	mg kg^{-1}	6000	3700	950		
Silica	mg kg^{-1}	34000	34000	5400		
Sulfur	mg kg^{-1}	860	1800	1000		
Lead	mg kg^{-1}	< 2	4	< 2	< 120	< 150
Cadmium	mg kg^{-1}	< 0.2	< 0.2	< 0.2	< 1.5	< 1.5
Copper	mg kg^{-1}	30	19	16	< 100	< 100
Nickel	mg kg^{-1}	5	14	12	< 30	< 50
Mercury	mg kg^{-1}	< 0.07	< 0.07	< 0.07	< 1	< 1
Zinc	mg kg^{-1}	120	61	39	< 400	< 400
Chromium	mg kg^{-1}	7	15	14	< 80	< 90
Boron	mg kg^{-1}	74	10	< 1		
Manganese	mg kg^{-1}	210	300	200		

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high, which could indicate zinc accumulation by the Eupatorium plants, as other sources of contamination can probably be excluded.

The most toxic compound among the PAH-16 used as benchmarks by the environmental authorities in many countries is benzo(a)pyrene. Concentrations of benzo(a)pyrene were 0.01–0.06 mg/kg (Table 1), well below the Norwegian maximum tolerable risk (MTR) level for soils where 95% of art diversity is protected (0.5 mg/kg)[41]. In addition, PAHs in biochar are only very sparingly bioavailable, often less than 1% [28]. Due most probably to the optimized outgassing under the fire front the PAH EPA16 contents were low (2.3 to 6.6 mg kg^{-1}). However, while both water quenched metal cone biochars would qualify for EBC premium quality (< 4 ±2 mg kg^{-1}), the soil snuffed biochar would only entitle for basic quality (< 12±4 mg kg^{-1}). It can be assumed that the hot water vapor that penetrates from bottom to top through the biochar layers during the water quenching process has an activating effect and may expulse PAH containing gases out of the biochar pores [42]. This activating and tar reducing effect can also be seen in the nearly 50% higher specific surface area of the water quenched eupatorium char (215 $m^2 g^{-1}$) compared to the soil snuffed char (149 $m^2 g^{-1}$).

The thermogravimetric analysis (TGA) (S2 Fig) of $BC_{E\text{-}soil}$ and $BC_{E\text{-}wood}$ showed that for both biochars the highest treatment temperature (HTT) was in between 680° and 750°C. The curb between 150° and 550°C showed a rather regular continuum of volatile organic carbon (VOC) release indicating a rather complete pyrolysis (no uncharred particles) and homogenous cooling at the end of the pyrolysis process. Interestingly, the pure eupatorium biochar has slightly lower VOC content (64% vs 70% at HTT) probably due to the smaller particle size of the eupatorium feedstock and thus faster heat conduction, faster pyrolysis and better vapor penetration during water quenching.

Overall, the three representative biochars produced in flame curtain kilns were of high quality comparable with high-tech produced higher temperature biochars [34, 43] and all qualifying for the EBC certificate which is the baseline for authorization to use biochar as soil amendment in e.g. Switzerland and Austria. Moreover, $BC_{E\text{-}met}$ was already tested in an agronomic field trial in Nepal and proved its plant growth enhancing potential by increasing the pumpkin yield fourfold when blended with cow urine and compost and more than doubled when blended only with compost both compared to the control which was amended only with compost and cow urine [24].

Gas emission factors

Emission factors were, in g per kg biochar produced: CO 54 ± 35, CH_4 30 ± 59, TSP 11 ± 15, NMVOC 6 ± 3, NO_x 0.4 ± 0.3, and total products of incomplete combustion, PIC, 100 ± 83. These data are based on 17 runs of 10 to 15 data points each, totaling around 250 individual measurements per gas/aerosol. The high standard deviations thus do not reflect a lack of data but rather a high variability of gas emissions during individual kiln runs. This variability is caused by variations in burning conditions during the individual runs: e.g. if the flame curtain is interrupted by putting on too much feedstock, pyrolysis gases are not completely combusted and spikes in gas emissions are observed. In addition, the above-mentioned variations in biochar yield influence the emission factors in g per kg biochar. Especially the methane emission data (Table 3) had large standard deviations: methane concentrations were mostly below the limit of detection of 0.1% (around 10 g/kg biochar), whereas they occasionally leaped up to 1–3% (100–300 g/kg char). Such spikes coincided with events where much of the flame curtain was absent due to feeding with too much feedstock, underscoring that the flame curtain is pivotal to sustain low emissions.

Fig 1 compares the average emission factors for the flame curtain kilns (n = 17) with values that were previously measured for traditional and retort kilns. For the comparison to retort kilns only values from Sparrevik et al. [12] were used because these measurements were carried out with exactly the same equipment and measuring methodology and because we dispose of the complete series of data for these measurements. For the comparison to traditional kilns, data from [12] and [10] were used. Overall, the data were based on eight runs for traditional kilns, and five runs for retort kilns.

The flame curtain kilns had significantly lower emissions of CO and NO_x (54 ± 35 and 0.4 ± 0.3 g/kg biochar, respectively) than traditional or retort kilns (CO: 351 ± 141 and 148 ± 64 g/kg biochar, respectively; NO_x: 2.0 ± 1.6 and 1.7 ± 1.0 g/kg biochar, respectively). The total products of incomplete combustion (PIC) emissions of the flame curtain kilns were significantly lower than those of non-retort and retort kilns. Non-methane volatile organic

Table 3. Emission factors (g/kg charcoal) of CO_2, CO, CH_4, TSP [aerosols, from particulate matter < 10 μm (PM_{10})], non-methane volatile organic carbon (NMVOC), and the sum of nitrogen oxide and nitrogen dioxide (NO_x), as well as the sum of all products of incomplete combustion, PIC (all gases except CO_2). Average values per flame curtain kiln type and per feedstock, and kiln literature values (traditional non-improved kilns, retort kilns with syngas circulation and combustion, TLUDs).

		n[a]	CO_2	CO	NMVOC	CH_4	TSP	PIC	NO
Per flame curtain kiln type									
All-Steel deep octagonal	this study	n = 3	5600 ± 700	38 ± 20	6 ± 2	57 ± 52	22 ± 28	123 ± 82	0.3 ± 0.1
Steel-shield Soil pit	this study	n = 3	2300 ± 800	23 ± 28	5 ± 5	14 ± 20	9 ± 7	51 ± 31	0.3 ± 0.2
Soil pit	this study	n = 3	3800 ± 1300	36 ± 40	8 ± 1	32 ± 44	20 ± 24	97 ± 108	0.8 ± 0.7
shallow steel pyramidal and octagonal	this study	n = 10	4700 ± 800	73 ± 31	5 ± 3	26 ± 75[b]	5 ± 4	108 ± 93	0.32 ± 0.12
Per feedstock type									
100% Eupatorium	this study	n = 9	4600 ± 2100	74 ± 34	6 ± 3	60 ± 90[b]	11 ± 16	151 ± 109	0.4 ± 0.2
80% Eup, 20% wood	this study	n = 3	3400 ± 2300	23 ± 26	5 ± 3	28 ± 34	23 ± 27	79 ± 89	0.1 ± 0.2
50% Eup, 50% wood	this study	n = 3	3900 ± 2000	13 ± 4	9 ± 1	13 ± 21[c]	9 ± 7	43 ± 25	0.7 ± 0.6
50% Eup, 50% Rice husk	this study	n = 2	3810 ± 50	47 ± 16	3.0 ± 0.2	0	3 ± 2	52 ± 19	0.260 ± 0.002
Kiln literature									
Traditional kiln	Ref. [10, 12][d]	n = 8[e]	2375	351	53	49	19	472	2.2
Retort kiln	Ref. [10, 12][d]	n = 5[e]	2602	148	7	35	11	202	1.7
TLUD	Ref. [20]	n = 5[e]	n.r.	94	274	40	7	415	0.0
High-tech large-scale reactor	Ref. [44]		3010	$3{\cdot}10^{-7}$	0	0	0.05	0.05	0.7

[a] n is number of datasets (time series during one kiln run). Each dataset consists of 10–15 measurements. Thus, the total number of measurements is 20 to 150.

[b] large std since value is dominated by one large value of 238 g/kg char.

[c] large std since value is dominated by one large value of 37 g/kg char.

[d] average of two literature datasets where each data set was given equal weight.

[e] one dataset per kiln type.

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carbon (NMVOC) emissions were significantly lower for flame curtain kilns (6 ± 3 g/kg biochar) than for traditional kilns (53 ± 4 g/kg biochar). Methane and TSP emissions were not significantly different between the flame curtain, traditional and retort kilns. CO_2 emissions were significantly higher for the flame curtain kilns than for retort or traditional kilns, which is a direct consequence of the slightly lower yields and lower non CO_2-emissions obtained in flame curtain kilns. CO_2 is the lowest caloric and least climate hazardous emission product of biomass combustion and a measure of the completeness of the combustion of pyrolytic gases. PIC, the sum of all C-containing products of incomplete combustion, is dominated by CO (around 30 to 70%), and thus PIC could be lower for flame curtain kilns than for retort and traditional ones, even though TSP (< 20% of the total PIC) was not.

In flame curtain pyrolysis the combustion of the main pyrolysis gases appears to be fairly complete due to efficient and turbulent mixing of these gases with combustion air above the pyrolysis zone. However, the heat and combustion dynamic is apparently not sufficient to completely combust less inflammable aerosols (TSP). For that reason TSP rates were comparable to retort kilns while the emission of the more ignitable pyrolysis gases like CO, and NMVOC was significantly lower.

The currently measured emission factors were comparable to literature values for TLUD stoves (Table 3), with the exception of NMVOC, where literature values are approximately one order of magnitude higher than the values for flame curtain pyrolysis. The similarity in gas and TSP emission factors between flame curtain kilns and TLUD stoves was expected because of the similar principle of pyrolysis gas combustion, where pyrolytic gases are formed below a

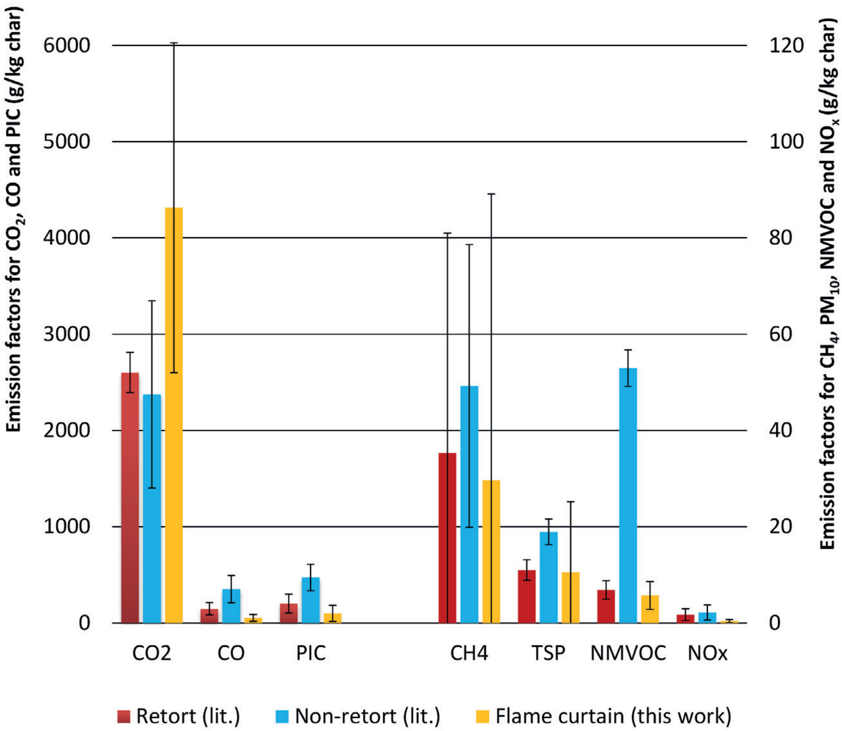

Fig 1. Emission factors for CO_2, CO, CH_4, TSP (aerosols, derived from PM_{10} as described in the methods, non-methane volatile organic carbon (NMVOC), and the sum of nitrogen oxide and nitrogen dioxide (NO_x), as well as the sum of all products of incomplete combustion, PIC (all C-containing gases except CO_2). Flame curtain: based on 17 runs of 10 to 15 measurements each done within the present study. Retort and non-retort kilns: average values from refs. [10, 12]. Error bars represent standard deviations in 50 to 250 individual measurements.

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flame, carried upwards by the up-draft and subsequently combusted in the flame, suppressing the emission of combustible gases and particles such as CO, methane, NMVOC and aerosols.

The lower yields, higher CO_2 emissions and lower CO emissions for flame curtain kilns compared to traditional or retort kilns are explained by the principle of the open flame curtain: close to the high temperature of the open flames more feedstock gasifies and these pyrolytic gases combust more completely which results in lower yields, higher CO_2 emissions and lower combustible emissions like CO, CH_4 and others.

Various flame curtain kiln subtypes

Differences between the various subtypes of flame curtain kilns or various feedstocks were non-significant in all cases except CO_2 emissions from steel-shielded soil pit kilns (2300 ± 800 g/kg biochar) being lower than those of all-steel deep cone kilns (5600 ± 700 g/kg biochar) (Table 3). This result is encouraging in the sense that simple conically shaped soil pit flame curtain kilns, if they are operated properly, result in biochar yields, C contents and gas / aerosol emissions that are similar to those of the all-steel deep conical flame curtain kilns. This implies that high-quality biochar can be made in a sustainable manner without investing more than for the labor involved in digging out the soil pit, drying the feedstock and carrying out the pyrolysis.

For the two runs done with 50% rice husk and 50% Eupatorium, emission factors did not significantly differ from those for eupatorium or eupatorium/wood mixtures (Table 3). It should be noted though that the timing of the layer placement during pyrolysis is more crucial for the rice husk than for the other feedstocks because if too much of the low-densityrice husks are added too quick and/or at once, the flames are snuffed which leads to higher emissions especially of methane and aerosols. Since the performed rice husk runs were executed by a skilled operator, such emissions were not observed here.

Implications

In Table 4, the advantages and disadvantages of various medium-size kiln types are compared. The biochar yield of 22 ± 5%, which is somewhat lower than that of retort kilns [12, 13], is a disadvantage of flame curtain kilns. This is not a significant hindrance in the case of biochar for soil amendment made from low-value organic residues like shrubs, straw and husks which are materials that cannot be pyrolysed in such retort system without a large portion of valuable fire wood. However, it is an important factor to consider in the case of charcoal making from high-value wood for cooking purposes, where yields need to be high in order to reduce deforestation and increase the economic value of the charcoal making activity.

The flame curtain kiln offers multiple advantages:

1. gas and aerosol emissions are relatively low (for CO even lower than those of retort kilns) compared to other small scale biochar and charcoal production technologies but not to large-scale processes (Table 3);
2. no wood is required for startup;
3. construction and operation is much easier and more economic compared to retort kilns;
4. pyrolysis is much faster (hours) than in most traditional and retort kilns (days). The process might actually be too fast for the complete pyrolysis of thick wood logs in shallow kilns when thinner materials are mixed in; in case of charcoal making from wood logs, it is advised to use well-insulated deep cone kilns, to use only wood as feedstock, to finalize with thinner branches at the top and snuff with soil or rather iron lid instead of quenching with water;

Table 4. Advantages and disadvantages of various medium-size kiln types.

	Application	Main advantages	Main disadvantages
Biochar-generating TLUD cookstove	Kitchen gardens, cooking purposes	Energy for cooking, Saving firewood, Low gas emission factors	Too small to generate larger amounts of biochar
Traditional kilns	Agriculture, charcoal making	Familiarity, Low investment cost, Complete pyrolysis of thicker logs	High gas emission factors, Slow (4 days)
Retort kilns	Agriculture (possibly + energy), charcoal/ briquette making	Lower emissions than traditional kilns, High biochar yield, Energy generation possible with pyrolysis heat, Complete pyrolysis of thicker logs	High investment cost, Startup wood required, Complicated construction and operation, Slow (2 days)
Flame Curtain Kilns	Agriculture + heat, charcoal making (small logs)	Relatively low emissions esp. of CO, No startup wood required, Easy to construct and operate, Fast (3 hours for 1 m^3 biochar), Low to zero investment cost, Heat recovery	Relatively low biochar yield (charcoal making), Incomplete pyrolysis of thick logs
Power-generating systems	Energy + agriculture, briquette making	Power generation, Negligible emissions	Relatively high investment cost, Low caloric content of briquettes

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5. heat from pyrolysis gas combustion can easily be recovered for drying, distillation, hot water production or cooking;
6. investment costs are low (for the steel deep cone kilns) to negligible (for the conically shaped soil pit kilns). The last argument might be decisive for tropical farmers on the poorest soils where biochar possibly has the strongest positive agricultural effects: as these farmers need to sustain on meager yields grown on these difficult soils, they often do not have the resources to invest in novel technologies. In the case of charcoal making for cooking purposes, the flame curtain kilns are certainly more sustainable than the also free earth-mound kilns, because of the advantages mentioned above especially the lower gas/aerosol emissions.

The quality of the flame curtain kiln biochars was good with regard to all relevant parameter for EBC and IBI certification and showed further high CEC and SSA values (Tables 1 and 2). Pyrolysis temperatures of the flame curtain kilns (700°C) are higher than those of traditional or retort technologies (400–500°C)[10, 12], and this results in a more porous and more condensed biochar [45]. Higher porosity certainly implies stronger contaminant immobilization [46] and probably also higher nutrient retention [47]. More condensed higher-temperature biochars exhibit higher H/C_{org} ratios which have been related to relatively strong N_2O emissions reductions upon their amendment to soil in a recent meta-analysis [48]. Finally, in another meta-analysis higher-temperature chars have tentatively been associated with negative priming, i.e., increases in soil organic matter upon the amendment of biochar to soil [49]. Overall, in many cases the high-temperature flame curtain chars can be expected to be of higher quality than lower-temperature ones made by traditional technologies, depending on the purpose the respective biochar or charcoal is intended for.

Conclusion

The Kon-Tiki flame curtain pyrolysis is a new type of low cost biochar and charcoal production technology with pyrolysis gas combustion. It can easily be built and used by farmers both in the developed and developing world. It was shown that the quality of biochar produced from various feedstocks complies with international quality standards like IBI and EBC. Gas and aerosol emissions were very low compared to all other low cost and traditional charcoal and biochar production devices.

Supporting Information

S1 Description. Gas Analyses. Experimental details of gas emission analyses.
(DOCX)

S2 Description. Carbon balance and emission factors. Carbon balance and emission factors: accurate description of the calculation of carbon balance and gas emission factors.
(DOCX)

S1 Fig. Kiln types. Overview of kiln types tested in this paper.
(DOCX)

S2 Fig. TGA analyses. TGA analyses of two representative biochars ($BC_{E\text{-}soil}$ and $BC_{E\text{-}wood}$). Temperature was ramped from 25 to 950°C in 2 hours. "Gewichtsverlust" is loss of weight (both rate and overall loss), "Zeit" is time.
(DOCX)

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Author Contributions

Conceived and designed the experiments: GC MS HPS. Performed the experiments: GC HPS NRP BHP. Analyzed the data: GC HPS. Contributed reagents/materials/analysis tools: MS. Wrote the paper: GC MS HPS. Designed the kilns: HPS PT.

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S1 Fig. Kiln types. Overview of kiln types tested in this paper.

	All-steel deep octagonal kiln Octagonal deep cone kiln without rim shield. Upper long diagonal: 1500 mm Lower long diagonal: 820 mm Depth: 980 mm Outer angle: 70°
	All-steel shallow octagonal kiln Oct 55° Octagonal shallow cone kiln without rim shield. Upper long diagonal: 1131 mm Lower long diagonal: 381 mm Depth: 400 mm Outer angle: 55°
	All-steel pyramid kiln Pyr 55° Upper long diagonal: 1100 mm Lower long diagonal: 382 mm Depth: 400 mm Outer angle: 55°

	All-steel shallow pyramid kiln Pyr 45° Upper long diagonal: 1740 mm Lower long diagonal: 382 mm Depth: 480 mm Outer angle: 45°
	Conical soil pit Upper diameter: 1500 mm Lower diameter: 800 mm Depth: 900 mm Outer angle: 60-70°
	Steel-shielded soil pit Upper diameter: 1500 mm Lower diameter: 800 mm Depth: 900 mm Outer angle: 70°

 	Snuffing of soil pit kiln with soil, pressed with feet and shovel to make it air-tight.
	Quenching with water or nutrient liquids If water pressure is sufficient, the water is pumped into the kiln from the bottom of the kiln; the fire is then extinguished only at the end of the quenching process with some water dousing from the top avoiding thus that pyrolysis gases escape unburned. Alternatively, quenching water is introduced at one side only until the water level reaches the top.

	Measurement of the gas emissions using a chimney to channel the escaping gases so that they could be measured simultaneously.

S1 Description. Gas Analyses. Experimental details of gas emission analyses

A Microtector II 6460 was used to analyze carbon dioxide (CO_2) and methane (CH_4), both with a detection limit of 0.1% by infrared sensors and non-methane volatile organic components (NMVOC) with a detection limit of 0.1 ppm by photoionization detection (PID). The PID was calibrated using isobutene. Carbon monoxide (CO) and nitric oxide (NO) were analyzed with a Kigaz 300 flue gas analyzer by internal jacket type electrochemical sensors. Detection limits was 1 ppm for both sensors. For CO values above 8000 ppm the Kigaz instrument internally dilutes the gas stream to be able to measure concentrations up to 50 000 ppm. The instrument converts NO to generic nitric oxides (NO_x) by applying a conversion factor of 1.03, thus assuming that 97% of NOx consists of NO. Particles in the form of PM_{10} were analyzed with a Thermo Scientific pdr-1500 instrument by use of photometric detection of particles (detection limit 0.1 μg/m^3). In fact the particles below 2.5 μm are the most carcinogenic to humans (Smith and Mehta, 2003). PM_{10} and $PM_{2.5}$ have found to be well-correlated in some studies (Wang et al., 2006) but less so in others (Castillejos, 2000). In our case, we selected PM_{10} over $PM_{2.5}$ since we then could avoid cyclones and pre-filtration, which may introduce unnecessary measure errors when working with direct measurements of exhaust gases.

For conversion of concentration from mass units to molar ratios in the particle measurements, all particles were assumed to consist of elementary carbon. For subsequent conversion from TSP to total suspended particles (PM_{10}) a conversion factor of 1.4 was used, thus assuming around 70% content of PM_{10} in the samples (Schikowski et al., 2005). Based on the measurements the value for products of incomplete combustion (PIC) were given by summarizing the values for CO+NMVOC+CH_4 and TSP. All sensors except the particle analyzer were protected by a 0.45µm particle filter that was changed regularly during measurements. Polytetrafluoroethylene (PTFE) tubing was used for sampling. Readings were taken as composite samples from the chimneys of the kilns during the pyrolysis process. Between three and ten readings were taken during the process depending on the duration of the charring. The samplings always included start-up and operational mode (retort mode when relevant). The cooling processes were not sampled since hardly any gases are emitted from the material during this period and thus their inclusion would result in skewed data. For all gases and readings, a molar ratio between the component and CO_2 was calculated. From these single-point ratios, a time-weighted average (TWA) was calculated between each subsequent measurement point to be representative for this specific period of the process. The different portions were then integrated over the whole process period and a grand mean value representative for the whole carbonization process was calculated. Since the process is proceeding in different stages (including switching from non-retort to retort mode in the retort kilns) TWA is better representative for the process than the use of geometric mean values.

S2 Description. Carbon balance and emission factors. Carbon balance and emission factors: accurate description of the calculation of carbon balance and gas emission factors.

In order to calculate the emission factors of the kilns the widely used carbon balance method was utilized (Bailis et al., 2003; Pennise et al., 2001; Zhang et al., 2000). In this method, only the emission ratios between the gases are measured without the need to register the absolute mass of gases emitted. Instead, this mass is calculated by performing a carbon balance between the biomass entering the process and the biochar produced. Thus, the difference in carbon was assumed equal to the mass of carbon in the emitted gases. The molar ratios were then used to calculate the distribution of carbonaceous gases in the emitted smoke. For open systems like the present ones, the carbon balance method is preferable over absolute measurements of gas composition because controlling all gases escaping from the process is challenging.

Adapted from Zhang et al (Zhang et al., 2000), the mass balance of carbon in feedstock combustion process can be described as follows;

$$C_{feedstock} - C_{char} = C_{CO_2} + C_{CO} + C_{CH_4} + C_{NMVOC} + C_{TSP} \quad (1)$$

where $C_{feedstock}$ is the carbon content in the biomass feedstock and C_{char} is the carbon content in the processed biochar material on the left side of equation (1). On the right side is the sum of all combustion gases containing carbon.

Rearranging eq. (1) yields;

$$\frac{C_{feedstock} - C_{Char}}{C_{CO_2}} = 1 + \frac{C_{CO}}{C_{CO_2}} + \frac{C_{CH_4}}{C_{CO_2}} + \frac{C_{NMVOC}}{C_{CO_2}} + \frac{C_{TSP}}{C_{CO_2}}$$

(2)

K can be defined as the sum of all emission ratios of the components to CO_2

$$K = \frac{C_{CO}}{C_{CO_2}} + \frac{C_{CH_4}}{C_{CO_2}} + \frac{C_{NMVOC}}{C_{CO_2}} + \frac{C_{TSP}}{C_{CO_2}}, \quad (3)$$

Subsequently 1+K can be defined to represent all products of pyrolysis including CO2.

The emission factor of CO_2 on a carbon basis is defined as mass of emissions pr. mass of char produced (m_{char}):

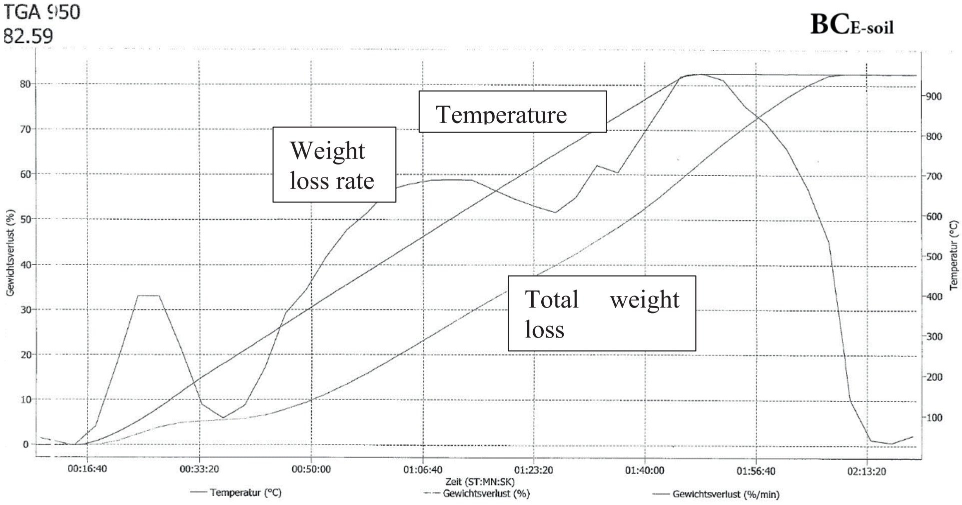
$$E_{m,CO_2-C} = \frac{C_{CO_2}}{m_{char}} = \frac{C_{feedstock} - C_{char}}{(1+K)m_{char}} \quad (4)$$

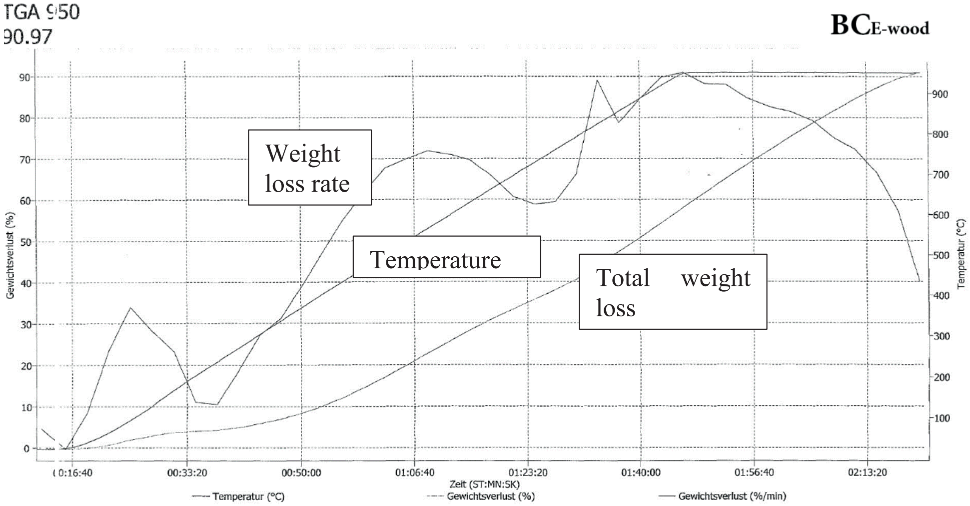
By solving equation (4), the mass of CO_2-C can be calculated and converted to CO_2 by using the C/CO_2 molar ratio. The other gases of interest can be found by their respective molar ratios to CO_2-C.

After the calculation of the mass of all gaseous components, the emission ratio can be related either to the amount of char or to the amount of C produced. In the present study, we did not achieve significant amounts of visibly identifiable brads or ash (measured in a previous study with the same biochars showing around 10% ash (Hale et al., 2013)). Bio oil was attempted to be collected on surfaces as lids and drums after pyrolysis but the amounts were insignificant (<1% of the biochar mass produced). The emissions factors were therefore solely based on the weighing of all the produced material, which we defined as biochar.

The collection of gas emission data under rural conditions and at different field sites in various countries is a time-consuming and difficult process (Pennise et al., 2001). Even though multiple measurements of gases during the individual runs substantiated a correct representation of the emissions in that specific run, uncertainties may still be present since; i) kilns are different in construction, ii) a wide array of feedstocks can be used, and iii) the kilns were operated by local people using their operational practices. We addressed this by pooling the data together into two kiln types (retort and non-retort) and applying statistical analysis to conclude on the validity of the results especially sensitivity to use of different feedstocks. A two sample t-test using the statistical package SPSS statistics version 21 was used to test for effects of kiln type (retort and non-retort) on; i) biochar yield, ii) molar ratios and finally on iii) emission factors (CO_2, CO, VOC, CH_4, TSP, PIC and NO).

S2 Fig. TGA analyses. TGA analyses of two representative biochars ($BC_{E\text{-soil}}$ and $BC_{E\text{-wood}}$). Temperature was ramped from 25 to 950 ºC in 2 hours. "Gewichtsverlust" is loss of weight (both rate and overall loss), "Zeit" is time.





Paper II.

Biochar from "Kon Tiki" flame curtain and other Kilns: Effects of Nutrient Enrichment and Kiln Type on Crop Yield and Soil Chemistry

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Hans Peter Schmidt, Gerard Cornelissen

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RESEARCH ARTICLE

Biochar from "Kon Tiki" flame curtain and other kilns: Effects of nutrient enrichment and kiln type on crop yield and soil chemistry

Naba Raj Pandit[1,2], Jan Mulder[1], Sarah Elisabeth Hale[3], Hans Peter Schmidt[4], Gerard Cornelissen[1,3]*

1 Institute for Environmental Sciences (IMV), Norwegian University of Life sciences (NMBU), Ås, Norway, **2** Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal, **3** Norwegian Geotechnical Institute (NGI), Oslo, Norway, **4** Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, Arbaz, Switzerland

* gco@ngi.no

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Abstract

Biochar application to soils has been investigated as a means of improving soil fertility and mitigating climate change through soil carbon sequestration. In the present work, the invasive shrub "*Eupatorium adenophorum*" was utilized as a sustainable feedstock for making biochar under different pyrolysis conditions in Nepal. Biochar was produced using several different types of kilns; four sub types of flame curtain kilns (deep-cone metal kiln, steel shielded soil pit, conical soil pit and steel small cone), brick-made traditional kiln, traditional earth-mound kiln and top lift up draft (TLUD). The resultant biochars showed consistent pH (9.1 ± 0.3), cation exchange capacities (133 ± 37 $cmol_c\ kg^{-1}$), organic carbon contents (73.9 ± 6.4%) and surface areas (35 to 215 m^2/g) for all kiln types. A pot trial with maize was carried out to investigate the effect on maize biomass production of the biochars made with various kilns, applied at 1% and 4% dosages. Biochars were either pretreated with hot or cold mineral nutrient enrichment (mixing with a nutrient solution before or after cooling down, respectively), or added separately from the same nutrient dosages to the soil. Significantly higher CEC *($P < 0.05$)*, lower Al/Ca ratios *($P < 0.05$)*, and high OC% ($P<0.001$) were observed for both dosages of biochar as compared to non-amended control soils. Importantly, the study showed that biochar made by flame curtain kilns resulted in the same agronomic effect as biochar made by the other kilns ($P > 0.05$). At a dosage of 1% biochar, the hot nutrient-enriched biochar led to significant increases of 153% in above ground biomass production compared to cold nutrient-enriched biochar and 209% compared to biochar added separately from the nutrients. Liquid nutrient enhancement of biochar thus improved fertilizer effectiveness compared to separate application of biochar and fertilizer.

Introduction

Biochar (BC) is the carbon-rich material produced by the pyrolysis of biomass i.e. heating in the partial or complete absence of oxygen [1]. Biochar is highly recalcitrant in nature unlike other forms of soil organic matter (SOM). Thus, biochar amendment to soils acts as a carbon

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sequestration technique which can also enhance soil fertility [1–3]. Agronomic benefits of biochar-amended soils can be the result of improved soil physical properties (bulk density, porosity, water holding capacity, permeability, aggregation), biological properties (improved environment for microbial populations such as mycorrhizae) and chemical properties (pH, CEC and nutrient retention capacity) [4–11].

Various pyrolysis technologies and various feedstocks can be used to produce biochar. This may result in a large variation in resulting biochar properties [12,13] which in turn may affect biochar effectiveness for increasing soil fertility [14,15]. Low temperature pyrolysis (300–500˚C) has shown increased biochar yield and carbon content whereas high temperature pyrolysis (>500˚C) has revealed lower biochar yield and higher surface area with increased adsorption capacities for various compounds [16]. Research on the effect of pyrolysis technology on agronomic biochar quality has up until now been scarce. Under rural (sub)-tropical conditions, biochar has mostly been produced with medium-sized traditional kilns made of bricks or simple earth mound heaps, improved retort kilns [17,18] or top-lit up-draft (TLUD) pyrolysis units [19]. Traditional kilns can be operated using all kinds of mixed biomass feedstocks. However, pyrolysis gases such as methane (CH_4), carbon monoxide (CO) and aerosols (PM 2.5 and PM 10) are released untreated, and this leads to greenhouse gas emissions, pollutant emissions and loss of energy [20]. Improved retort kilns have features to recirculate the produced syngases into the combustion chamber, resulting in up to 75% less toxic and greenhouse gas emissions as well as higher conversion efficiency (40–50%) compared to traditional brick kiln, due to less losses of energy-rich molecules [21]. On the other hand, improved retort kilns are more costly, difficult to operate and often consume a lot of start-up biomass materials [18]. TLUD kilns burn feedstock cleanly, thereby reducing gas emissions, as the syngases are combusted largely in the flame front. If used indoors this reduces negative health impacts [22]. There are some limitations with using relatively small TLUDs as they produce so little biochar (around 300 g per run) that they are mainly useful for small-scale kitchen gardening [20]. Larger TLUDs, while generating more biochar, require significant investments and expertise in order to be operated successfully.

To circumvent such challenges, the flame curtain, open pit "Kon-Tiki" kiln was recently developed [23]. It follows the principle of pyrolyzing biomass layer after layer in an open, conically built metal kiln that is easy to operate, fast, and results in low greenhouse gas emissions [20]. It thus allows biochar production in relatively large quantities (700 to 850 L volume biochar in 4–5 hours) [20–23]. The flame curtain kiln can even be operated as a simple conically shaped hole in the ground, leading to the same low emissions and similar biochar quality as the metal version, but essentially without any cost apart from the few hours of labour required to dig and prepare the soil pit [20].

Most studies on weathered soils have shown significant positive effects of biochar application on crop production; however, other studies have not shown any significant or even negative effects of biochar on crop yield [24,25]. Some examples from tropical countries on mostly acidic and weathered soils include the following. Radish yield increased significantly in biochar amended soils blended with mineral N fertilizers in pot trials, emphasizing the role of biochar in improving nitrogen use efficiency [2]. Moreover, conservation farming practice carried out with 4 tons/ha of biochar in a maize field in Kaoma, Zambia characterized by sandy acidic soils result in strong increases (0.9 ± 0.1 t ha^- without biochar to 3.8 ± 0.5 t ha^- with biochar) in crop yield [26]. Furthermore, application of biochar at 10 t ha^{-1} along with NPK mineral fertilizers (50g m^{-2}) in maize, cowpea and peanut field showed an increase of 322%, 300% and 200% respectively compared with control plot (without biochar and NPK) in South Sumatra, Indonesia [7]. In contrast, field application of biochar did not show agronomic effects at four sites out of six in Zambia [26]. In seven field trials on five working farms in the UK, [27] observed positive yield effects in three trials, no effects in three trials and negative yield effects in one trial.

Recently, techniques for biochar nutrient enrichment, i.e. mixing nutrients with biochar before addition to the soil, have resulted in some promising increases in crop yield. Biochar enriched with cattle urine and amended to soil in Dhading, Nepal, increased the yield of pumpkin to 82.6 t ha^{-1} [28], more than 300% higher than that with only urine and 85% higher than the yield with the same amount of biochar without urine added. In another study, biochar enriched with compost nutrients by co-composting in the presence of biochar, was added to sandy soils and increased the yield of Chenopodium quinoa by 300% compared to non-enriched biochar treatments in the presence and absence of compost [29]. Biochar nutrient enrichment is probably effective due to penetration of nutrients in biochar micro- and nano-pores. The pores of carbonaceous sorbents such as biochar are so narrow that water movement is restricted and an ice-like water structure is formed [30]. Earlier work has provided evidence of a relation between organic compound sorption and the nanopore volume of such matrices [30] and it is possible that a similar phenomenon could occur for nutrients in biochar. Nutrient addition to biochar has thus shown to be a promising method to enrich the biochar and render it a slow-release fertilizer. However, systematic studies on the optimal way to carry out such nutrient enrichments are lacking.

This is the first study to directly compare the agronomic effect of biochar produced from different kiln types and enriched in different ways (enriched hot biochar and enriched cooled-down biochar, as compared to non-enriched biochar where the same amount of nutrients was added separately). The study was carried out using a pot trial design in Nepal using a woody shrub as biochar feedstock. "*Eupatorium adenophorum*" is a promising feedstock as it is a naturally regenerating, ubiquitous, invasive woody forest shrub species locally named "Banmara" (forest killer) that is about 1–2 m high and stems up to 2 cm thick [31]. In this way, waste from an invasive species can be turned into a valuable resource for agronomic production and carbon sequestration. Biochar produced from Eupatorium feedstock has been found to meet all the requirements for premium quality based on European Biochar certificate [20]. In Nepal, average landholding size is very small and the soils can be acidic, exhibiting lower levels of C, N, P and exchangeable bases [32]. Overall, this study tested the following hypotheses: (1) Biochar produced from various kilns with different pyrolysis conditions exhibits different crop yield effects depending on kiln type, and (2) Nutrient enrichment improves the agronomic effect of biochar thereby increasing the maize biomass production.

Materials and methods

Biochar

Biochar (BC) was produced using several different types of kilns; flame curtain kilns (four sub types: deep-cone metal kiln, steel-shielded soil pit, conical soil pit and steel small cone kiln), brick-made traditional kilns, traditional earth-mound kilns, and TLUD kilns. Photographs of each of these production methods are shown in the supporting information (Image A in S1 File) along with a description and principle of their operation (Description A in S1 File). The feedstock used for the generation of biochar was the woody shrub Eupatorium, which was collected from forests close to the site of pot trials at Matatirtha, Kathmandu, Nepal (N 27˚ 41' 51", E 85˚ 14' 0", altitude 1520 m). Stems were 1–2 cm thick. Eupatorium had 25% moisture content at the time of pyrolysis [20]. Elemental analysis of the Eupatorium was carried out using an *EuroEA Elemental Analyzer* and showed that the biomass contained 42.9% C, 1.4% H and 1.5% N. For the flame curtain kilns, Eupatorium was subjected to a maximal pyrolysis temperature of around 600˚C just below the flame curtain, as measured by an Impex digital thermometer with a 60-cm temperature-resistant sensor pin [20,23] cooling down to 200–400˚C as the pyrolyzing biomass was getting further and further down below the flame curtain upon the layer-by-layer addition of new feedstocks. Pyrolysis temperatures for the other kilns were lower, around 400 to 500˚C before final quenching with soil or water [17]. Following the pyrolysis process which took place over a period of around 2 hours per batch, biochars produced from the deep-cone metal flame curtain kiln, steel small cone, TLUD and brick kiln were quenched or snuffed with water whereas biochar produced from the steel shielded soil pit and conical soil pit flame curtain kilns were snuffed with soil (Image A in S1 File). Weight and volume of the biochar were measured after water snuffing and soil snuffing.

Biochar nutrient enrichment

Biochar was nutrient-enriched using two methods, namely hot and cold nutrient enrichment. Hot and cold nutrient enrichment refers to hot and cooled-down biochar, respectively, that were enriched with mineral fertilizers (NPK) added dissolved in water. Hot nutrient enrichment was carried out by pouring hot (200 to 400 C) biochar at the rate of 30 g and 120 g (equivalent to 1% (20 t ha^{-1}) and 4% (80 t ha^{-1} biochar respectively) in 1 L dissolved nutrients in a bucket. For both biochar rates, all biochar was submerged, however, biochar for the 1% amendments was enriched in a thinner slurry (higher liquid to solid ratio) than the biochar added at a 4% rate. During hot nutrient enrichment, the biochar was cooled down from 200–400 ^{0}C to < 40 ^{0}C upon contact with the nutrient solution. The nutrient solution contained urea, di-ammonium phosphate (DAP) and potash as the source of nitrogen (N), phosphorous (P) and potassium (K) respectively. Urea, DAP and potash was used at the rate of 5.11 g pot^{-1}, 2.34 g pot^{-1} and 1.8 g pot^{-1} which is equivalent to 2.7g pot^{-1} N, 1.08 g pot^{-1} P and 1.08 g pot^{-1} K. The lukewarm mixture in the bucket was then stirred thoroughly for 10 minutes to ensure the biochar was well mixed with the solution. Cold nutrient enrichment was carried out using a similar method with the same volume of water and amount of NPK but adding biochar that was water quenched and cooled down beforehand. After enrichment, the bucket was sealed and the biochar allowed to rest for 10 days. The liquid remaining that was not absorbed by the biochar was later added to the respective treatment pot to ensure the same fertilizer dose addition to each respective pot.

Soil

The soil used for the pot trial was taken from a field at Rasuwa farmland (27^{0}, 59,479' N and 85^{0}, 11.987' E, altitude 1365m). The study was conducted on private farmland. No specific permission apart from that from the farmer was required for these locations to take the composite soil sample. The exiting field trials in Rasuwa did not involve endangered or protected species. The soil was collected from 0–30 cm depth and was well homogenized by repeated shoveling. The soil was an inceptisol (order) having low soil pH of 4.5 and base saturation of less than 50% [33].

Pot trial

A pot trial was carried out in order to investigate the effect of different biochars, produced using different methods and enriched in different ways (hot mineral nutrient-enriched, cold mineral nutrient-enriched and non-enriched biochar) had on soil characteristics and crop production. The pot trial was carried out in June-July 2015 in a greenhouse located in Matatirtha, Kathmandu, Nepal. The average daily temperature for the time period when the pot trial was carried out were 22^{0} C (minimum 15 ^{0} C and maximum 29 ^{0} C). However, temperatures in the greenhouse were higher than those values (minimum 20 ^{0} C and maximum 49 ^{0} C). Nursery plant pots (25cm top diameter and 25 cm height) were filled with 3 kg dry soil. Biochar (dry or slurry, dependent on treatment) was added to the pots at two different doses; 1

and 4% biochar (approximately 20 t and 80 t biochar ha^{-1}) based on dry soil and biochar weight and were mixed until completely homogeneous.

Seven different kiln types (7 levels), three mineral nutrient enrichment techniques (hot mineral nutrient enrichment, cold mineral nutrient enrichment and non-enrichment) each with 1% and 4% biochar dosages (6 levels) and their interaction with kiln type and nutrient enrichment techniques along with two controls illustrated 21 treatments/levels (N = 86) in total (Table 1). For biochar produced from flame curtain deep cone metal kilns and traditional brick kilns, two dosages of biochar (1% and 4% biochar) were used for hot mineral nutrient enrichment, cold mineral nutrient enrichment and non-enrichment (biochar separately added to the soil), leading to a total of 12 treatments for these production methods. For the TLUD produced biochar, the same two dosages of biochar were used, but the biochar was not enriched. For the conical soil pit, steel shielded soil pit and traditional kiln production methods, only one dosage (4%) of biochar (not enriched) was used (Table 1). In addition to these biochar additions (Table 1), two control treatments i.e. control (C1) without biochar and without NPK (non-fertilized control, n = 4) and a control (C2) with only mineral fertilizer (fertilized control, n = 5) were also used.

Two maize seeds were initially sown 2 cm below the soil surface in each pot. Upon germination and emergence of two leaves (after 12 days), the smaller plant, selected based on visual observation, was removed from the pot to leave one plant for the experimental duration. Each pot was watered daily with 0.7 L (corresponding to 20 mm rainfall) water. Pots were arranged in randomized complete block design (RCBD) comprising five blocks/replications. Pots in each block were rotated at an 8-day interval to ensure the homogeneity of the treatments. Weeding was carried out 20 d (1st weeding) and 35 d (2nd weeding) after sowing.

Biochar, soil and maize plant analyses

Maize plants were harvested after 50 d and were separated into above ground biomass (AGB which comprised the shoot) and below ground biomass (BGB which comprised the root), just

Table 1. Treatments to test biochar quality variations with (i) kiln type, and (ii) nutrient enrichment type and iii) interaction of kiln type and nutrient enrichment type biochar. These biochar type consists of 19 levels (N = 77) with two additional control treatments C1 and C2 (N = 9) where all biochar amended treatments (19 levels, N = 77) were compared with these control treatments (N = 9). The numbers T1 to T21 correspond to different treatments number with its respective replications (n = 3, 4 or 5, N = 86) in parentheses.

		Nutrient enrichment type					
		1% BC hot mineral nutrient enrichment	**4% BC hot mineral nutrient enrichment**	**1% BC cold mineral nutrient enrichment**	**4% BC cold mineral nutrient enrichment**	**1% BC non-enriched**	**4% BC non-enriched**
Kiln type	Traditional brick kiln	T1 (n = 5)	T2 (n = 4)	T3 (n = 5)	T4 (n = 3)	T5 (n = 4)	T6 (n = 3)
	Deep cone metal kiln	T7 (n = 5)	T8 (n = 4)	T9 (n = 4)	T10 (n = 5)	T11 (n = 4)	T12 (n = 3)
	Small cone kiln	-	-	-	-	T13 (n = 5)	T14 (n = 4)
	TLUD (top lift up draft)	-	-	-	-	T15 (n = 4)	T16 (n = 4)
	Conical soil pit	-	-	-	-	-	T17 (n = 3)
	Steel shielded soil pit	-	-	-	-	-	T18 (n = 3)
	Traditional earth mound	-	-	-	-	-	T19 (n = 5)
Control	Non-fertilized control (C1)	T20 (n = 4)					
	Fertilized control (C2)	T21 (n = 5)					

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above the brace roots. Both AGB and BGB fresh weight were measured immediately after harvesting. Roots were washed carefully with clean water. Plant biomass (AGB and BGB) was oven dried at 70 ^{0}C for 24 hours for dry weight analyses.

Soil samples were collected after harvesting of maize plants. Soil from all individual replicate pots was collected to make a composite sample for each of the 21 treatments. Soil analyses were conducted both prior and after the amendment of the biochar, i.e., in the presence and absence of biochar. The biochar-amended soils were analyzed after the experiment (various biochar amended treatment soils). Soil samples were oven dried at 40 ^{0}C for three days and passed through a 2mm sieve and ground (< 2mm) prior to analysis. Sieved samples were used for determining pH and cation exchange capacity (CEC) and ground samples were used for total carbon, nitrogen and hydrogen (CHN) analysis. Soil pH was measured in both 0.01M $CaCl_2$ and in water (soil: solution ratio of 1:2.5 in volume basis) using an Orion 1 Ross pH electrode. Total CHN was measured by elemental analysis using an *EuroEA Elemental Analyzer*. For CEC measurement, NH_4NO_3 extractable cations were extracted by adding 25ml 1M NH_4NO_3 to 3g soil, gently shaken and kept overnight. The suspension was transferred to 250ml volumetric flask through the funnel with washed blue ribbon filters (Whatman 589/3) until 250ml was collected. 15ml of 1M NH_4NO_3 extracted solution was poured in 15ml ICP tubes (Inductively Coupled Plasma) to measure the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}). For H^+ determinations, the 1M NH_4NO_3 extraction solutions (20ml) were titrated with 0.05 M NaOH.

Biochar generated from the different kilns was collected after production. Biochar samples were treated in the same way as soil samples and analyzed for pH, CEC and total CHN. BET surface area was determined by N_2 adsorption at 77 K using an automated surface area analyzer. The samples were outgassed by heating at 110o C under a flow of ultrahigh purity helium at 10 cm^3min^{-1} for 16 to 24 h prior to analysis. Isotherm data were recorded at partial N_2 pressures of 0.03 to 0.7 atm. The apparent surface areas of samples were obtained from the statistical monolayer capacities of N2 from the BET plots [34]. Because of the risk of N losses as NH_3, the concentration of N absorbed to the char was measured in the study. Since P and K through volatilization can be ruled out, these nutrients were not analyzed in the enriched chars. For mineral N (N_{min}) analysis, (NO_3^- and NH_4^+) in char, biochar sample operating hot mineral nutrient enrichment was collected. N_{min} analysis was performed through standard 2M KCl extraction methods.

Statistical analysis

Data were statistically analyzed using R software (R version 3.2.2, R commander 2.2–1) and excel. Data normality was checked prior to performing linear model ANOVA analysis. Two factor linear ANOVA model was used to explore the effect of kiln type's biochar (7 levels) and mineral nutrient enrichment techniques (hot, cold and non-enriched) including both dosages of biochar (6 levels) and their interactions (19 levels) on maize biomass yield (dry AGB, height and node diameter) (Table 1). Biochar produced from different kiln types and three different mineral nutrient enrichment types (19 levels) were compared with non-fertilized and fertilized control treatments (2 levels) via one way ANOVA. Significant effect observed in the ANOVA were further explored through Post Hoc Tukey test to compare all the treatment means and their significance against each other on maize biomass production. Soil samples were pooled per treatment for statistical analysis where the effect of biochar amended soils i.e. 1% biochar (n = 8) and 4% biochar (n = 11) on soil pH, CEC, Ca/Al and total CHN content were compared with non-fertilized and fertilized control soils.

Results

Biochar yield and properties

As earlier reported [20], average biochar yields from Eupatorium feedstock on dry weight basis and carbon basis were 19.5 ± 5.0% and 40.2 ± 10.1%, respectively (Table A in S1 File). These numbers were in the same order of magnitude as those for biochar from various other kiln techniques at various pyrolysis temperatures [35–38].

Chemical analysis of biochar samples showed a consistent pH of 10.12 ± 0.19 (H_2O extraction) and 9.11 ± 0.27 ($CaCl_2$ solution), which showed that variation in pyrolysis temperature between flame curtain kilns and traditional methods did not influence the pH of biochar. On average, biochars produced from different kilns all had relatively high CECs of 133.3 ± 37.2 $cmol_c\ kg^{-1}$. Total C, H and N content of biochar samples produced from different kiln types were 73.9 ± 6.4%, 1.81 ± 0.43% and 0.74 ± 0.16% respectively. Average surface areas (SA) of biochar samples were 97 m^2/g, ranging from 35.4 to 215 m^2/g (Table A in S1 File). These results show that alkaline biochar with high CEC, C content and SA was produced independent of the various production methods tested in this work (the novel flame curtain, TLUD, traditional brick and earth-mound kiln).

N analysis (NO_3—N) of hot nutrient-enriched biochar showed 1.08 ± 0.12 mg NO_3^- kg^{-1} biochar and 0.81 ± 0.02 mg NO_3^- kg^{-1} biochar for the biochar added at 1% and 4% respectively (Table B in S1 File). Similarly, 313 ± 5.77 mg NH_4^+ kg^{-1} biochar and 120 mg NH_4^+ kg^{-1} biochar was observed for hot nutrient-enriched biochar to be added at 1% and 4% dosages, respectively (Table B in S1 File). These N_{min} contents were likely underestimated as only one singular KCl extraction was done while Kammann et al [29] and Haider et al [39] have recently demonstrated that serial KCl extractions of biochar may lead to significant higher N_{min} quantities captured by biochar. Total N contents for hot nutrient-enriched biochar were 4.3% and 2.5%, respectively, for the biochar to be added at 1% and 4% dosages. Based on the amount of nutrients in the enrichment solution, it could be calculated that between 50 and 100% of the added N was retrieved in the biochar (Table B in S1 File).

Biochar effect on soil properties

The tested Rasuwa soil was sandy and acidic with low pH (4.5), CEC (12.3 cmolc/kg) and organic carbon (OC; 1.5%). Biochar-amended soils showed increased average pH (4.84 ± 0.50) compared with the fertilized control soil (4.30 ± 0.02) (Table C in S1 File). Average Al/Ca ratios after addition of 1% biochar dose (0.18 ± 0.06) and 4% biochar dose (0.03 ± 0.04) were significantly lower ($p < 0.05$) than those of non-fertilized (0.30 ± 0.04) and fertilized (0.36 ± 0.08) control soils (Fig 1). Absolute exchangeable Al (III) contents of the unamended soils (0.8 to 1.0 $cmol_c$/kg) were within the range where toxic Al effects on plant roots can be expected [6,26]. Average CEC after amendment with 1% and 4% biochar dosages were 17.1 ± 0.1 $cmol_c$/kg and 29.5 ± 5.1 $cmol_c$/kg, respectively, significantly higher ($P< 0.05$) than those of non-fertilized (11.2 ± 0.7 cmolc/kg) and fertilized (12.1 ± 0.4 cmolc/kg) control soils (Fig 1, Table C in S1 File). The increase in CEC was higher than expected on the basis of additivity, which is probably caused by the pH effect of biochar, resulting in an increase in CEC measured by extraction with non-buffered NH_4NO_3 solution. Also soil organic carbon (SOC) contents with the 1% biochar dose (1.9 ± 0.1%) and the 4% biochar dose (3.3 ± 0.4%) were significantly higher ($P<0.001$) than those of control treatments (1.5 ± 0.1%) (Fig 1). However, addition of biochar (70% C) for 1% and 4% biochar dosages to soil containing 1.5% SOC should have resulted in around 2.2% SOC and 4.3% SOC on the basis of pure additivity, which was higher than the measured values of 1.9% and 3.3% SOC, respectively. Hence, in contrast to CEC, the

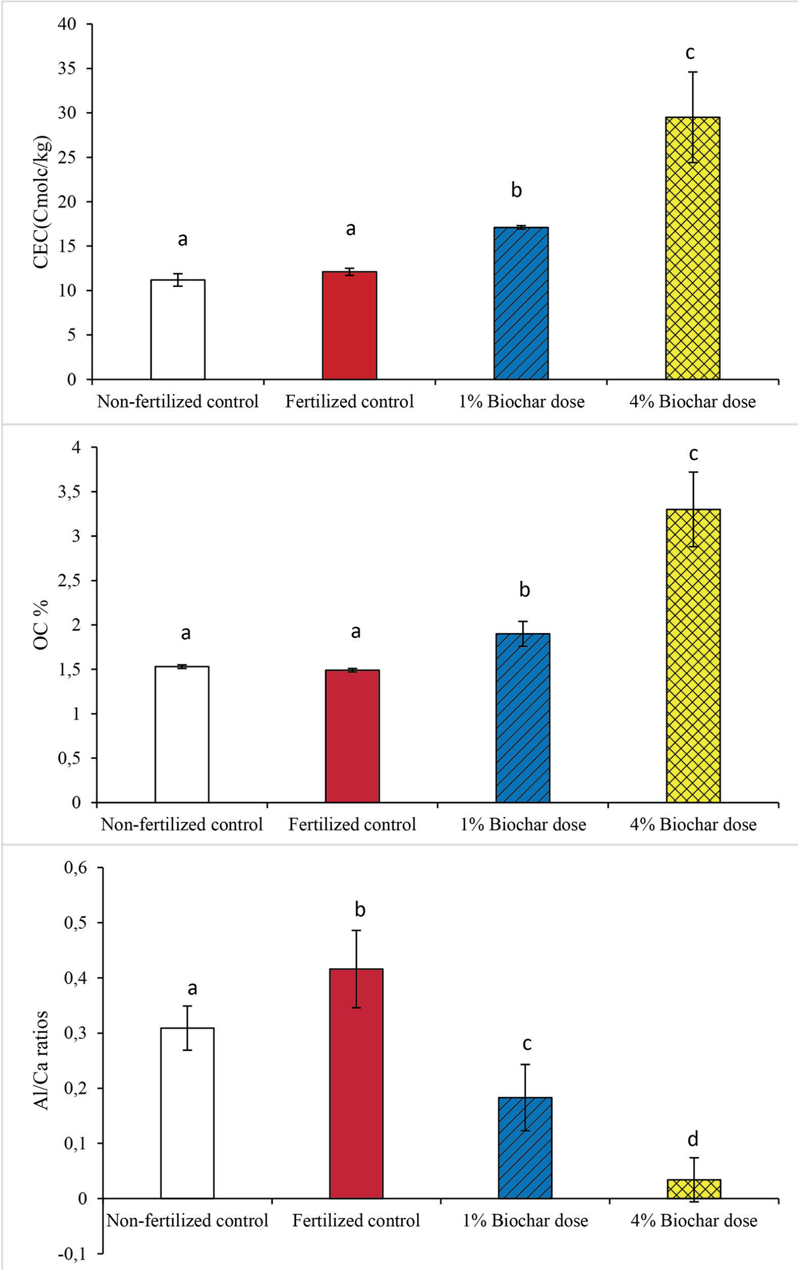

Fig 1. Effect of biochar dosage (1% and 4% biochar) on soil CEC, OC% and Al/Ca ratios. Biochar produced from different kiln either hot or cold mineral nutrient enrichment or non-enriched were pooled together

for the statistical analysis to assess the effect of biochar dosages (1% and 4% biochar) and non/fertilized control (without biochar) on soil properties. Average CEC, OC% and Al/Ca ratio plotted on y-axis and 1% biochar dose (n = 9), 4% biochar dose (n = 11) and fertilized and non-fertilized control (n = 2) treatments were plotted against x-axis. Significance codes (a, b. . ..) were provided based on t-test at 0.05 level of significance.

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amount of SOC in the biochar-amended soil was less than expected on the amount of C added via the biochar. There were no significant variations between biochar properties arising from the use of different kilns. Thus, the improved soil chemical properties were the result of biochar addition irrespective of pyrolysis technique.

Maize biomass production in biochar vs. non-biochar soils

Before comparing the effect of the different types of biochar production method and the nutrient enrichment techniques on crop yield, we present the results of the overall effect of biochar amendment on maize biomass production. All biochar amended treatments (21 levels, N = 86) revealed significant effect ($P<0.0001$) on maize biomass production. This was expressed by both maize above ground biomass (AGB) ($P<0.0001$), maize height ($P<0.0001$) and, to a lesser extent, maize node diameter ($P<0.0001$). Among all biochar amended soils, AGB production increased most with 1% hot mineral nutrient enriched biochar produced from traditional brick kiln (+ 248%) and produced from deep cone metal kiln (+168%), respectively, compared with fertilized control (Fig 2). Similarly, 4% biochar produced from traditional brick kiln and deep cone metal kiln encompassing hot mineral nutrient enrichment increased AGB production to 176% and 223%, respectively, of the values of fertilized control pots (Fig 2). Average maize dry AGB production per pot as a main effect of 1% biochar dosage and 4% biochar dosage increased to 165% and 139% ($P < 0.001$) respectively of the values of the fertilized control soils without biochar (Fig A in S1 File). Similar trends were found for maize height and node diameter. Lowest maize biomass production (3.02 ± 0.29 g pot^{-1}) was observed for non-fertilized control compared with biochar amended and fertilized control treatments (Fig 2, Fig 3, Table D in S1 File).

Effect of biochar made with different kiln types on maize biomass

Biochar produced from seven different kiln types did not show significant variation in maize biomass production (dry AGB, height and node diameter) (Table 2). When the various kiln methods were compared to each other, maize AGB production did not show significant variation for both non-enriched biochar (produced from all seven different kiln types tested) and nutrient enriched biochar (produced from traditional brick kiln and flame curtain deep cone metal kiln; the only kiln types for which biochar enrichment was tested) (Fig D in S1 File). Thus, the agronomic effect of the flame curtain kiln biochar was similar to that of the other kiln types. On average for all kiln types, maize AGB, height and node diameter for non-enriched biochar were 4.7 ± 0.7 g, 54.7 ± 6.4 cm and 2.0 ± 0.3 cm respectively (Table D panel A in S1 File). On average for both kiln types (flame curtain and traditional brick kiln), nutrient-enriched biochar showed average maize dry AGB, height and node diameter of 8.6 ± 4.0 g, 78.5 ± 26.5 cm and 3.0 ± 0.8 cm respectively (Table E panel B in S1 File). Hence, biochar generation technique had no effect on maize biomass production, but nutrient enrichment had.

Effect of nutrient enrichment of biochar on maize biomass

Nutrient enrichment showed significant effects ($P<0.0001$) on maize biomass production (Table 2). Biochar hot nutrient enrichment at 1% dosage showed increases in average maize

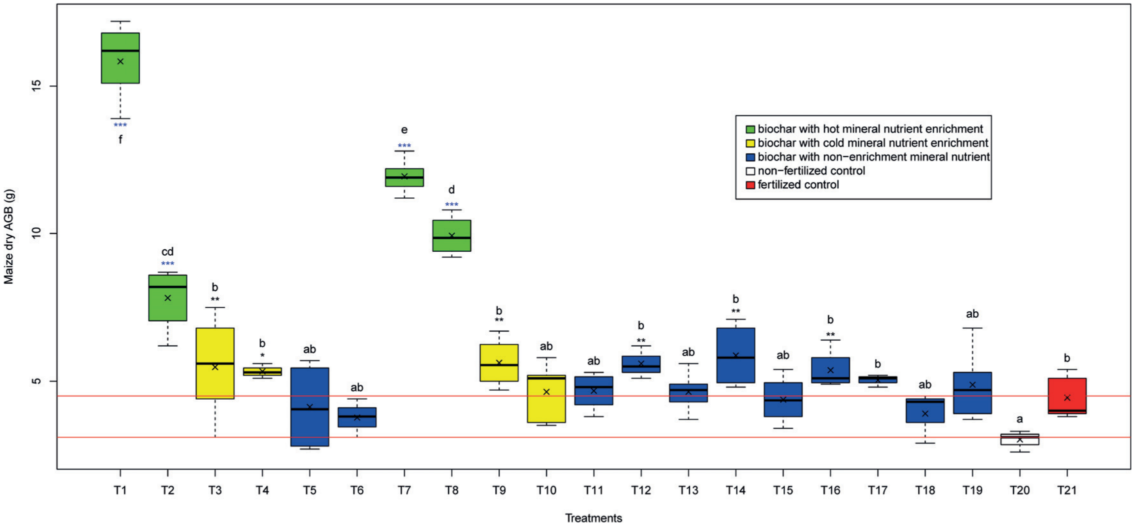

Fig 2. Effect of biochar amended soils produced from different kiln and enriched and non-enriched in various ways with 1 and 4% biochar dosages (19 levels; T1, T2. . .T19) vs fertilized and non-fertilized control treatments (2 levels; T20 and T21) on maize above ground biomass. Description of treatments (T1, T2. . .T21) is mentioned in the Table 1 and Table D in S1 File. Sign (x) in the middle of the box plot refer to the average maize AGB of each treatments. Asterisk (*) at the top of the box plot denotes the significant difference between biochar treatments over control (C1/T20 for non-fertilized control and C2/T21 for fertilized control) treatments (*** < 0.001, ** <0.01 and * <0.05 significance). Blue color asterisk (*) represents significance level for both non-fertilized (no color fill) and fertilized control (red color box plot) whereas black color (*) only for non-fertilized control (C1/T20). Different letters above box plot (a, b, c) represent significant differences between the treatments (T1 to T21).

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AGB of +153% and +209% of the values observed for cold nutrient-enriched and non-enriched biochar respectively, at the same dosage of biochar and nutrients, the nutrients having been added separately for the non-enriched biochars (Fig 3). Similarly, the higher 4%-dosage hot nutrient enriched biochar showed higher (*P<0.001*) average AGB than the 4% non-enriched (+82%) and cold-enriched biochars (+62%)(Fig 3). The study also showed that 1% hot nutrient enriched biochar amendment gave significantly higher maize biomass (*P<0.0001*) than all of the 4% biochar treatments (hot nutrient enrichment, cold nutrient enrichment and non-enriched) (Fig 3). Similar trends were observed for maize height and maize node diameter (Figs E and F in S1 File). Overall, both dosages of biochar treated via hot nutrient enrichment showed significantly stronger effects on biomass yield (*P<0.0001*) compared to cold nutrient enriched biochar, non-enriched biochar and fertilized control treatments.

Interaction of kiln type and nutrient enrichment of biochar

The interaction of two factors: kiln type and mineral nutrient enrichment type for both biochar dosages showed significant effects (*P<0.001*) on maize biomass production (Table 2, bottom row). 1% biochar hot nutrient enriched produced from flame curtain deep cone metal kiln and traditional brick kiln showed higher biomass yield (*P<0.001)* compared with 1% non-enriched biochar produced from flame curtain deep cone metal kiln, traditional brick kiln, steel small cone kiln and TLUD (Fig 2, Fig G in S1 File). In contrast, 1% cold nutrient enriched biochar did not show significant effect with 1% non-enriched biochar on maize biomass yield. Furthermore, there was no significant difference between 4% biochar (non-enriched)

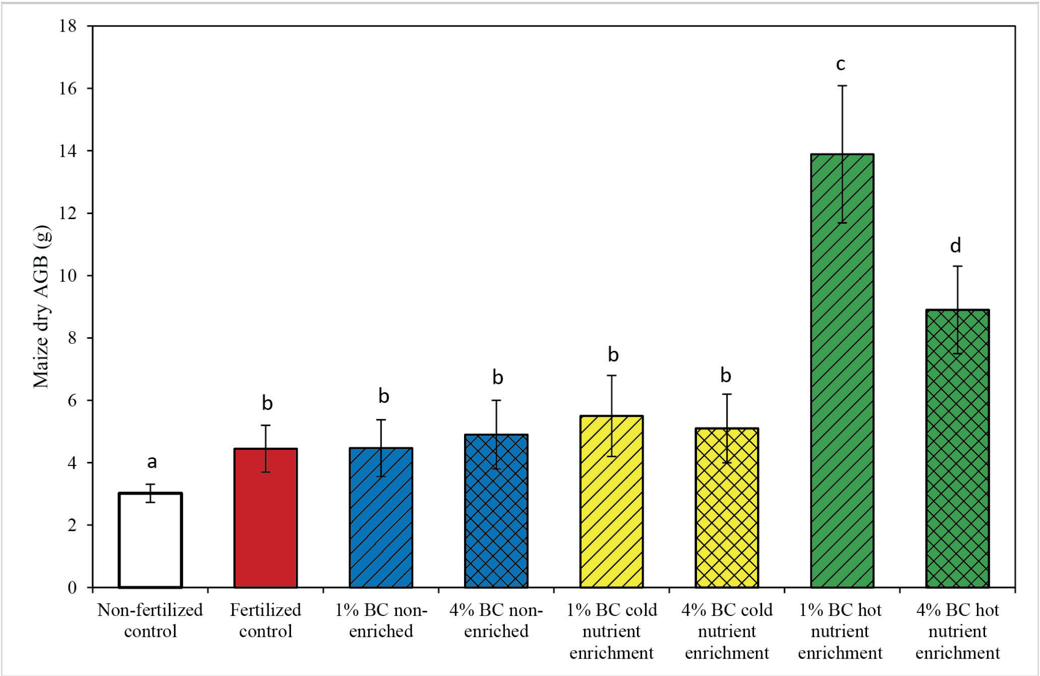

Fig 3. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize dry AGB yield (g). Maize dry AGB (g) is plotted as a function of mineral nutrient enrichment technique, along with two controls. Different letters above the bars (a, b, c) represent significant differences between mineral nutrient enrichment types and controls.

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produced from various kilns and 4% cold nutrient enriched biochar but a significant difference on biomass yield was observed between 4% non-enriched biochar versus 4% hot nutrient enriched biochar produced from flame curtain deep cone metal kiln and traditional brick kiln (Fig 2, Fig G in S1 File).

Discussion

Biochar and its effect on soil properties

In this study, the chemical properties of pure biochar produced from "*Eupatorium adenophorum*" via flame curtain kilns were in line with those reported by Schmidt et al, 2015 [28]

Table 2. Statistical analysis of two factor ANOVA (kiln type and mineral nutrient enrichment type's biochar) on maize biomass yield (N = 77). The table output corresponds to Fig D in S1 File for the effect of kiln type biochar, Fig 3 for nutrient enrichment type biochar and Fig 2 and Fig G in S1 File for the interaction between kiln type and nutrient enrichment type biochar on maize above ground biomass production (gm).

	Maize dry AGB (g)		Maize height (cm)		Maize node diameter (cm)	
Factor	***f-value***	***P***	***f-value***	***P***	***f-value***	***P***
Kiln type	*1.2*	*> 0.1*	*1.4*	*> 0.1*	*2.3*	*> 0.05*
Nutrient enrichment	*123.4*	*< 0.0001*	*104.5*	*<0.0001*	*24.9*	*< 0.0001*
Kiln type and nutrient enrichment type	*7.5*	*< 0.001*	*3.5*	*< 0.01*	*1.3*	*> 0.01*

https://doi.org/10.1371/journal.pone.0176378.t002 that used the same feedstock and kiln type biochars qualifying for premium quality of the European Biochar Certificate (EBC). This was the first study where the agronomic effect of biochar produced by flame curtain kilns was compared to that produced via other kilns (traditional brick kiln, TLUD and earth mound kiln). Alkaline biochar (pH 9), when applied to acidic soil, was shown to improve soil chemical properties (pH, CEC and SOC) and reduce deleterious available Al concentration (Table C in S1 File). Increases of soil pH, CEC and SOC were in line with results from earlier studies on sandy and/or acidic soils [40–42].

For SOC, a lower increase was observed than that expected on the basis of additivity (i.e., the amount of C added via the two biochar dosages). This may be due to one or several of these four reasons; i) heterogeneity in soil samples; ii) oxidation of biochar C; iii) leaching of soil or biochar dissolved organic carbon (DOC) [43], or iv) leaching of microscopic biochar particles [44]. Mechanisms (ii) and (iv) were favored by the green house conditions (high temperature and daily irrigation); mechanism (iii) was favored by the increase in alkalinity leading to DOC losses [43]. Biochar is commonly touted for its ability to sequester organic carbon (low C mineralization) for several years [45], however, temperature and moisture availability greatly affects the SOC retention and losses [46]. Biochar stability (CO_2 sequestered over a 100 year perspective) estimated from literature H/C ratios [47] for the biochar produced from various kiln reported 78% (earth mound kilns), 77% (retort kilns) and 90% (flame curtain kilns, TLUDs, gasifiers) in accordance with differences in operation temperature being lower for earth-mound kiln and retort kilns than for other three kiln types (Table F in S1 File). Thus, freshly produced biochar is not a completely inert material and part of it is prone to oxidation in contact with soil [48]. For example, Hamer et al. reported that C losses depends on feedstock biomass type where biochar produced from corn stover and rye was decomposed more quickly than wood [49].

Kiln type biochar and its effect on maize biomass production

In this study, the maize biomass production obtained with amendments with biochar made by flame curtain kilns was not shown to be significant different from maize biomass with biochar made with the other kiln types (Table 2, Fig D in S1 File), either non-enriched or enriched. This falsified hypothesis (1), and was corroborated by the observation that kiln type did not result in significant variation in biochar characteristics such as CEC, pH and OC content [50]. Even though flame curtain kilns showed lower emission factors and higher biochar production efficiencies [20], and are operated at higher temperatures, none of the four different flame curtain kilns showed biochar chemical properties (Table A in S1 File) and crop biomass production (Fig D in S1 File) that significantly differed from those observed for biochar generated by the other kilns. In accordance with this, Deal et al [50] reported no variation in biochar characteristics (pH, CEC and OC) produced from different kiln types/pyrolysis temperatures.

Similar non-significant trends of crop yield with kiln type (different pyrolysis conditions) were observed for the biochar produced from ponderosa pine and macadamia nut feedstock under slow and fast pyrolysis types for perennial grass, *Koeleria macrantha* [51] and lettuce/maize corn [52], respectively. Furthermore, biochar produced from traditional kiln type (slow pyrolysis) with rice husk did not show significant effects on rice yield [53].

So far, there have not been any studies that have compared the agronomic effect of biochar produced by various kiln types. Further research on the influence of kiln type on biochar effectiveness for soil and crop yield is thus needed [54]. Soil quality and crop responses generally depend on biochar properties that in turn depend on pyrolysis temperature [55]. Biochar produced from both low and high temperature pyrolysis has shown improvement of soil chemical properties [6,9,40], however, these effects differ greatly dependent on soil mineralogy and types [56]. Without directly comparing kiln types in the same study, crop production in response to biochar produced from different kiln types operated at different temperatures has shown a wide range of effects, from positive to no differences or even negative yield effects [55]. In accordance with our findings for acid soils, meta-analysis showed that increases in crop yield upon biochar amendment were larger for acid soils than for neutral ones (26). However, in contrast to our findings, the authors reported a large variation with biochar properties and, implicitly, kiln types.

Nutrient enrichment of biochar and its effect on maize biomass

In order to investigate appropriate techniques of mineral nutrient enrichment of biochar, a pot trial was conducted where hot and cold biochar were enriched with liquid mineral fertilizer or applied separately with mineral fertilizer (non-enriched) in acidic soils, all with the same total amount of fertilizer. Nutrient enrichment could be an effective method to improve soil fertility because nutrients become reversibly trapped in the nano/micropores inside the biochar matrix where water movement is restricted, and act as a slow-release fertilizer, reducing nutrient leaching on low CEC soils [42,57]. This is the first study in which hot and cold nutrient enrichment have been compared. Hot nutrient enrichment showed better effects on crop yield than cold nutrient enrichment or separate addition of biochar and nutrients, confirming hypothesis (2).

An explanation why hot nutrient enrichment was more effective than cold nutrient enrichment can possibly be obtained by analogy with organic compound diffusion through soil and black carbon nanopores. The penetration of nutrients into biochar nanopores is most likely an activated process that probably takes place faster at increased temperatures: retarded nanopore diffusion of organic compounds is a highly activated process with activation enthalpies ranging from 60 to 100 kJ/mol [58]. This implies that the retarded pore diffusion rates, and thus the rates of nanopore penetration, increase by approximately a factor of 2 for each 10˚C increase in temperature (58). Thus we speculate that pore penetration in hot biochar (e.g., between 60 and 100˚C, the expected temperature range when 100–200˚C hot biochar is brought into water) could be 100–10,000 times faster than that at room temperature, analogous with observations for organic molecules in black carbon pores that showed 100 times faster diffusion at 60 C than 20˚C [59,60].

More research has to be done to explain the underlying nutrient enrichment mechanisms, including nutrient speciation and location on the microscopic level [61], and their effects on crop production. One of the few studies explicitly studying nutrient enrichment of biochar is by Kammann et al [29] who observed that co-composting of biochar enriched the material with nitrate and phosphate. The captured nitrate was largely protected against leaching and partly plant-available. The authors hypothesized that nitrate-water bonding in micro- and nano-pores was the mechanism of nitrate capture in biochar particles.

On the other hand, there is a significant volume of literature showing the nutrient retention ability of biochar [62]. For example, Ventura et al. [63] showed in a field experiment that NO_3^- leaching was reduced by 75% by the addition of 10 t ha^{-1} biochar, whereas NH_4^+ leaching was low and not influenced. Also Laird et al [64] observed that 2% biochar reduced total N and total dissolved P leaching from manure-added nutrients by 11% and 69%, respectively.

With regard to the speciation of N nutrients added to biochar, X-ray Photoelectron Spectroscopy (XPS) analysis and SEM imaging of co-composted biochars indicated the presence of iron oxide compounds and amine-NH_3 on the surface and pores of the biochars (61). Changes in N functional groups on the biochar surface upon composting indicated sorption and/or reaction with other N species [61].

Based on our study, we suggest not to extinguish the hot biochar by adding NPK solution to it just after pyrolysis, since this would lead to excessive N losses as NH_3 due to biochar's alkaline reaction (NH_4^+ can be deprotonated to NH_3, upon which gaseous losses of N can occur in combination with excessive temperature (200–400˚C) (unpublished field observations). Under field conditions, NH_3 losses upon the addition of urea solution to hot biochar in flame curtain kilns were observed by a strong ammonia smell. It is recommended to first dissolve the NPK in water to which hot biochar can be added after pyrolysis, when temperatures are between 100 and 200˚C. These data confirm the research conducted by Schmidt et al. 2015 [28], where biochar enriched with cattle urine showed significantly increased pumpkin yields, with an increase of 300% and 85% compared with only urine treatment and separate biochar and urine addition, respectively [28].

This study also showed that 1% hot nutrient enriched biochar gave significantly higher maize biomass ($P<0.0001$) than all of the 4% biochar treatments (hot nutrient enrichment, cold nutrient enrichment and non-enrichment) (Fig 3). This may be due to the fact that the addition of 4% biochar (corresponding to 80 t ha^{-1}), is a too high dosage, as has been observed before [41]. The amendment of 4% biochar is perhaps not realistic from a field perspective either and may result in too large alterations in other soil properties (physical, biological).

Conclusion

Biochar can be produced from the invasive plant species *"Eupatorium adenophorum"* using various different types of kilns. Among all kilns tested, flame curtain kilns showed the lowest gas emissions factors [20], however, the resulting biochar was observed to possess chemical properties and agronomic effect similar to those seen for biochars produced by other kiln types. A weathered soil (low pH, % C and CEC) with resulting low crop production was significantly improved resulting in increased maize biomass when biochar was amended to the soil in this greenhouse experiment. Biochar has shown improved soil chemical properties with increased soil pH, CEC, C and Ca/Al ratio in Nepalese acidic soils. The strongest effect was achieved after directly mixing the hot biochar with a nutrient (NPK) solution, rather than adding biochar and nutrients separately. Importantly, differences in agronomic and chemical quality between biochars generated by various technologies were small compared to differences between biochar nutrient enrichment methods.

Supporting information

S1 File. Image A. Kiln types. Overview of kiln types tested in this paper.
Description A. Biochar Production Technology through different kilns.
Table A. Properties of biochar.
Table B. Nitrogen content (NO_3-N and NH_4-N) of hot mineral nutrient (urea) enriched biochar substrate.
Table C. Soil properties of biochar amended and control soils.
Table D. Effect of kiln type biochar enriched with and without mineral nutrients on maize biomass after 50d.
Table E. Effect of kiln type on maize biomass yield after 50 d for non-enrichment biochar and enriched biochar.
Table F. Biochar stability calculated from literature H/C–ratios.
Fig A. Effect of 1% biochar dosage and 4% biochar dosages and both fertilized and non-fertilized control on maize dry AGB (g) production.
Fig B: Effect of biochar (BC) amended soils produced from different kiln and enriched and non-enriched in various ways with 1 and 4% dosages (19 levels) vs control treatments (2 levels)

on maize height.
Fig C: Effect of biochar (BC) amended soils produced from different kiln and enriched and non-enriched in various ways with 1 and 4% dosages (19 levels) vs control treatments (2 levels) on maize node diameter.
Fig D: Effect of kiln types biochar on maize dry AGB (g) production.
Fig E. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize height.
Fig F. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize node diameter.
Fig G. Effect of Kiln type biochar (1% and 4% dosages) enriched and non-enriched with mineral nutrient on maize biomass production.
(DOCX)

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Author Contributions

Conceptualization: GC JM SEH HPS NRP.

Data curation: NRP GC.

Formal analysis: NRP.

Funding acquisition: GC.

Investigation: NRP GC HPS.

Methodology: HPS GC JM SEH NRP.

Project administration: GC.

Resources: GC.

Software: NRP.

Supervision: GC JM HPS SEH.

Validation: JM SEH.

Visualization: NRP.

Writing – original draft: NRP GC.

Writing – review & editing: GC JM SEH HPS.

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Supplementary Information (SI)

Biochar from "Kon Tiki" flame curtain and other Kilns: Effects of Nutrient Enrichment and Kiln Type on Crop Yield and Soil Chemistry

Naba Raj Pandit[1,2], Jan Mulder[1], Sarah Hale[3], Hans Peter Schmidt[4], Gerard Cornelissen[1,3,]*

1) Institute for Environmental Sciences (IMV), Norwegian University of Life sciences (NMBU), Ås, Norway

2) Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal

3) Norwegian Geotechnical Institute (NGI), Oslo, Norway

4) Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, 1974 Arbaz, Switzerland

Image A:Biochar production technology (Images 1-7)

Biochar production technology where Eupatorium was used as the feedstock (images with description)

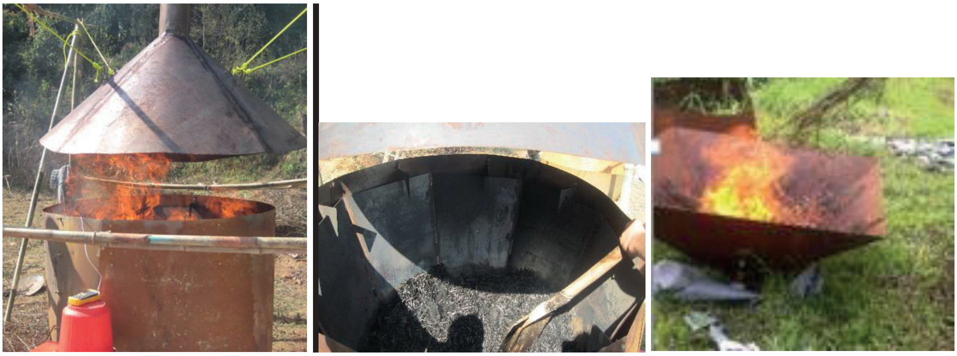

Image 1: Flame curtain deep cone metal kiln (left), biochar from flame curtain kiln (middle) and small metal cone kiln (right).

Image 2: Traditional brick kiln operation (left) and biochar harvested (right). Sizes : Inner wall; 1m x 1m x 1m and Outer wall; 1.4m x 1.4m x 1.4m.

Image 3: TLUD operation (left) and biochar harvested (right). Sizes: 0.65 m diameter, 1.05m height and 2.2m circumference.

Image 4: Steel shielded soil pit (left), Sizes: diameter (top: 1.02m, middle: 0.8m and bottom: 0.47m), height: 1.55m, and Conical soil pit (right), Sizes: diameter (top: 0.8m, bottom: 0.47m), height (0.8m).

Image 5: Traditional earth-mound kiln (1m^3 dimensions) practiced by farmers in Rasuwa (left) and BC harvested (right)

Image 6: Snuffing with soil (left) and water (right)

Image 7: Nutrient enriched biochar (left) and non-enriched biochar before mixing with soil (right)

Description A. Biochar Production Technology through different kilns

Flame curtain deep cone metal kiln and steel small cone Kiln

The fire was started on the top of the added biomass and the burning sparks ignite the feedstock at the bottom of the metal kiln which form the first layer of biomass. A thin bundle of Eupatorium (loosened) was added on the top of the sparks that heats quickly and starts to outgas. The biomass carbonizes beneath the flames due to low oxygen levels as the oxygen was consumed on the top. The next layer of eupatorium was added homogenously when ash appears on the outside of the carbonizing biomass. The combustion zone establishes a flame curtain that protects the biochar below from oxidizing. Feedstock was added continuously until the metal kiln was filled up and then quenched with water (1–3). The fresh biochar yield was measured immediately after quenching and the sample was collected for biochar dry matter analysis and characterization. For hot mineral nutrient enrichment, the hot biochar just after pyrolysis (700º C) was enriched in dissolved mineral fertilizer (NPK) in the form of urea (5.11 g), di-ammonium phosphate (2.34 g) and potash (1.8 g) containing at the rate of 500ml and 1 L water in the bucket of 10 l capacity for 1% BC and 4% biochar hot nutrient enrichment respectively. Cold nutrient enrichment was carried out in the same way (similar dose of NPK, dimension of a bucket and volume of water) but cooled-down biochar was used instead of still hot biochar.

Traditional Brick kiln

The inner and outer walls of the brick kiln are plastered with mud (Image 1). Feedstock (Eupatorium) is placed inside the brick kiln chamber and ignited. Bundles of feedstock are added until the kiln was filled up. After adding the last bundle, the top and bottom hole of the brick kiln are covered with corrugated tin plate for 2 hours without allowing oxygen to gets in. After two hours, the tin plate was removed and biochar was quenched with water and the yield was measured. Biochar sampling and characterization was operated in the same way as kontiki kiln BC.

Conical soil pit and steel-shielded soil pit

These kilns work according to the same principle as the flame curtain kiln. The only difference is that flame curtain "kontiki" (deep cone metal kiln and steel small cone) is made up of metal and conical soil pit and steel shielded soil pit was built under soil (Image 4). Feedstock was added layer after layer in the same way as that of kon-tiki until the hole was filled up and quenched with soil unlike kon-tiki. Biochar was harvested after 24 hours and yield was measured. Samples were collected for characterization.

TLUD (top lit up draft)

Eupatorium feedstock was kept inside the TLUD and ignited from the top. After the top portion started burning, the top was closed with the lid (Image 3). Pyrolysis was carried out for 18 minutes after which the lid was opened and quenched with water. Biochar yield was measured after quenching and biochar samples were collected.

Traditional earth-mound kiln

This kiln was adopted in Rasuwa district (Nepal) by rural farmers to produce a biochar. In this methods a 1 m^3 hole was dug and Eupatorium feedstock was burned layer after layer as mentioned for the conical soil pit. After the hole was filled up with added feedstocks layers, the last layer was quenched with soil. Yield was measured and the sample was collected for biochar characterization.

Table A. Properties of biochar. Biochar carbon yields, Cation exchange capacity (CEC), pH, surface areas (SA) and total C, H, N contents. Biochar produced from different kiln with 100% *Eupatorium* feedstock was either soil or water snuffed.

S.no	Biochar (Kiln type)	Quenching	Properties of Biochar								
			DM Yield (%)	pH		CEC (Cmolc/kg)	C (%)	H (%)	N (%)	C yield %	SA m^2/g
				Water	Cacl2						
1.	Traditional Brick kiln	Water	19	9.8	9.0	176	-	-	-	-	-
2.	Flame curtain- deep cone metal kiln	Water	18	9.9	8.7	121	77	-	-	36	84.9
3.	Flame curtain- All steel small cone										
3.1	Pyramid 45	Water	21	10.1	9.4	193	74.1	1.33	1.04	39	215
3.2	Pyramid 55	Water	17	10.0	9.0	100	77.2	2.01	0.72	32	72.9
3.3	Pyramid 60	Water	20	10.4	9.5	83	84.1	2.22	0.68	42	-
3.4	Pyramid 45 with shield	Water	27	10.1	9.5	217	72.5	-	-	49	-
3.5	Octagonal 60	Water	13	10.0	8.8		64.7	-	-	23	-
4	Flame curtain- Steel-shielded soil pit	Soil	25	10.0	8.9	121	81.2	-	-	56	35.4
							-	-	-		
5.	Flame curtain- Conical soil pit	Soil	18	10.4	9.2	127	71.4	2.16	0.66	43	74.6
							-	-	-		
6	TLUD	Water	12	10.2	9.0	95	63.5	1.35	0.62		-
7	Traditional earth-mound kiln	Soil	21	10.4	9.3	86	-	-	-		-

Table B: Nitrogen content (NO_3-N and NH_4-N) of hot mineral nutrient (urea) enriched biochar substrate. 5g hot enriched biochar sample extracted in 25ml 2M KCl and rest for 24 minutes for N characterization. 3.8 % N and 2% N was available as urea in 1% and 4 % biochar dosages being hot enriched. 0.5% N content was available in biochar itself; thus, with total nitrogen of 4.3% and 2.5% for 1% and 4% biochar hot enrichment respectively.

S.no	Biochar dosage hot mineral nutrient enrichment	NO_3-N (mg/kg), n=3	NH_4-N (mg/kg), (n=3)	C %	H %	N%	%TS
1	1% biochar dosages	1,08 ± 0,12	313 ± 5,77	76	1,7	4,3	12
2	4% biochar dosages	0,81 ± 0,02	120 ± 0	77	1,8	2,5	13

Table C. Soil properties of biochar amended and control soils. Biochar blended soils encompassed different kilns biochar, mineral nutrient enrichment and non-enrichment biochar that were applied in two (1% and 4% biochar) different dosages (n=19). Two additional control treatments (fertilized and non-fertilized, n=2).

Treatments	Kiln type biochar	Treatment (enrichment type)	pH		Ca/Al	CEC	Total CHN %		
			Water	$CaCl_2$		cmolc/kg	C (%)	H (%)	N (%)
Biochar amended soils	Traditional Brick kiln biochar	1% BC hot mineral nutrient enrichment	4,82	4,42	9,6	16,35	2,10	0,50	0,14
		4% BC hot mineral nutrient enrichment)	6,26	5,89	1000	45,66	3,65	0,47	0,18
		1% BC cold mineral nutrient enrichment	4,28	4,05	3,6	16,81	1,60	0,48	0,41
		4% BC cold mineral nutrient enrichment	4,69	4,59	65,2	35,29	2,98	0,49	0,19
		1% BC non-enriched	4,38	4,1	4,1	17,27	1,81	0,47	0,15
		4% BC non-enriched	5,55	5,32	12,6	18,89	3,40	0,49	0,18
	Flame curtain Deep cone metal kiln biochar	1% BC hot mineral nutrient enrichment	4,4	4,16	7,4	16,94	1,97	0,49	0,23
		4% BC hot mineral nutrient enrichment	4,48	4,32	28,6	28,41	3,17	0,49	0,21
		1% BC cold mineral nutrient enrichment	4,47	4,18	7,2	17,12	2,00	0,49	0,16
		4% BC cold mineral nutrient enrichment	5,17	4,87	100	22,44	3,02	0,50	0,18
		1% BC non-enriched	4,25	4,04	4,5	16,47	1,93	0,47	0,16
		4% BC non-enriched	4,67	4,48	23,3	23,98	3,65	0,51	0,19
	Steel small cone biochar	1% BC non-enriched	4,46	4,17	7,1	17,84	1,87	0,47	0,15
		4% BC non-enriched	5,47	5,28	311,1	26,18	3,85	0,50	0,18
	TLUD BC	1% BC non-enriched	4,33	4,06	5,7	16,95	1,92	0,50	0,14
		4% BC non-enriched	4,45	4,32	14,9	21,31	3,68	0,53	0,22
	Traditional earth-mound kiln biochar	4% BC non-enriched	4,28	4,13	6,5	21,04	2,85	0,47	0,17
	Steel shielded soil pit biochar	4% BC non-enriched	4,62	4,39	447.3	37,18	2,71	0,49	0,17
	Conical soil pit biochar	4% BC non-enriched	4,71	4,51	30,9	25,4	2,79	0,50	0,18
Control soils	Control (C1)	Non-fertilized control	4,57	4,19	3,2	11,19	1,53	0,49	**0,13**
	Control (C2)	Fertilized control	4,34	4,01	2,4	12,11	1,49	0,48	**0,22**

Table D: Effect of kiln type biochar enriched with and without mineral nutrients (19 levels) and control treatments (2 levels) on maize biomass after 50 d. Two factor ANOVA (kiln type and mineral nutrient enrichment biochar and it's interaction) includes 19 levels (T1-T19), N= 77; and one factor ANOVA includes 21 levels (T1-T21), N= 86.

ID code	Treatment type	Maize Biomass Production			
		Height (cm)	AGB (g)	Node diameter (cm)	(n)
T1	Traditional brick kiln 1% BC hot mineral nutrient enrichment	124.6 ± 4.72	15.84 ± 1.3	4.10 ± 0.3	5
T2	Traditional brick kiln 4% BC hot mineral nutrient enrichment	72.5 ± 5.12	7.82 ± 1.1	3.10 ± 0.6	4
T3	Traditional brick kiln 1% BC cold mineral nutrient enrichment	56.8 ± 13.1	5.48 ± 1.7	2.26 ± 0.7	5
T4	Traditional brick kiln 4% BC cold mineral nutrient enrichment	58.0 ± 3.6	5.33 ± 0.2	2.43 ± 0.1	3
T5	Traditional brick kiln 1% BC non-enriched	53.2 ± 10.7	4.12 ± 1.5	2.25 ± 0.4	4
T6	Traditional brick kiln 4% BC non-enriched	51.6 ± 3.2	3.76 ± 0.6	2.13 ± 0.2	3
T7	Deep cone metal kiln 1% BC hot mineral nutrient enrichment	107.0 ± 7.1	11.94 ± 0.6	3.46 ± 0.4	5
T8	Deep cone metal kiln 4% BC hot mineral nutrient enrichment	84.7 ± 5.6	9.92 ± 0.6	3.05 ± 0.3	4
T9	Deep cone metal kiln 1% cold mineral nutrient enrichment	59.7 ± 7.2	5.62 ± 0.8	2.40 ± 0.4	4
T10	Deep cone metal kiln 4 % cold mineral nutrient enrichment	54.0 ± 5.3	4.64 ± 1.1	1.88 ± 0.5	5
T11	Deep cone metal kiln 1% BC non-enriched	56.0 ± 5.2	4.67 ± 0.6	1.77 ± 0.3	4
T12	Deep cone metal kiln 4% BC non-enriched	60.6 ± 5.1	5.60 ± 0.5	2.20 ± 0.3	3
T13	Steel small cone kiln 1% BC non-enriched	55.6 ± 5.6	4.64 ± 0.7	1.74 ± 0.3	5
T14	Steel small cone kiln 4% BC non-enriched	64.5 ± 9.1	5.87 ± 1.1	3.46 ± 0.4	4
T15	TLUD 1% BC non-enriched	48.7 ± 6.2	4.37 ± 0.8	1.62 ± 0.3	4
T16	TLUD 4% BC non-enriched	53.7 ± 3.3	5.37 ± 0.6	1.80 ± 0.4	4
T17	Traditional earth-mound kiln 4% BC non-enriched	56.6 ± 2.3	5.03 ± 0.2	2.60 ± 0.3	3
T18	Steel shielded soil pit 4% BC non-enriched	51.0 ± 4.5	3.90 ± 0.8	1.56 ± 0.3	3
T19	Conical soil pit 4% BC non-enriched	54.4 ± 10.5	4.88 ± 1.2	1.94 ± 0.5	5
					N = 77
T20	Non-fertilized Control (C1)	44.2 ± 4.6	3.02 ± 0.2	1.25 ± 0.2	4
T21	Fertilized control (C2)	51.2 ± 4.6	4.44 ± 0.7	1.94 ± 0.2	5
					N= 86

Table E. Effect of kiln type on maize biomass production after 50 d for non-enrichment biochar (N=42) and enriched biochar (N=35)

A. Non-enrichment biochar (kiln types)				
Kiln types	**(n)**	**Average Biomass Production**		
		Maize height	**AGB**	**Node diameter**
Traditional brick kiln	7	52.5 ± 7.8	4.2 ± 1.0	2.2 ± 0.3
Deep cone metal kiln	7	58.0 ± 5.4	5.0 ± 0.7	1.9 ± 0.3
Small cone kiln	9	58.5 ± 8.4	5.0 ± 1.0	1.9 ± 0.4
TLUD	8	51.9 ± 5.4	4.8 ± 0.8	1.7 ± 0.3
Conical soil pit	5	54.4 ± 10.5	4.8 ± 1.2	1.9 ± 0.5
Steel shielded soil pit	3	51.0 ±4.5	4.3 ± 0.3	1.5 ± 0.3
Traditional earth mound kiln	3	56.6 ± 2.3	5.0 ± 0.2	2.6 ± 0.2
Average kiln type non-enriched biochar		**54.7 ± 6.4**	**4.7 ± 0.7**	**2.0 ± 0.3**
B. Enrichment biochar (kiln types)				
Traditional brick kiln	17	80.0 ± 30.3	9.1 ± 4.7	3.2 ± 0.9
Deep cone metal kiln	18	77.0 ± 23.1	8.1 ± 3.2	2.7 ± 0.7
Average kiln type (enriched biochar)		**78.5 ±26.5**	**8.6 ± 4.0**	**3.0 ± 0.8**

Table F. Biochar stability calculated from literature H/C-ratios according to Camps-Arbestain et al (4).

Production technology	**H/C-ratio**	**Stability (100 y)**	**Reference**
Earth-mound	**0,43 ± 0,15 (n=7)**	78%	Martinsen et al. [2] Obia et al. (in prep), Cornelissen et al. [3] Kupryianchyk et al. [4]
Retort	**0,44 ± 0,56 (n=2)**	77%	Kupryianchyk et al. [4]
Flame curtain	**0,22 ± 0,07 (n=14)**	90%	Schmidt et al. [5, 6] and in prep.
Gasifier/TLUD[1]	**0,28 ± 0,21 (n=4)**	90%	Shackley et al. [7]

[1] **The TLUD stove was assumed to give biochar with same stability as produced with a gasifier as the operation principle is the same**

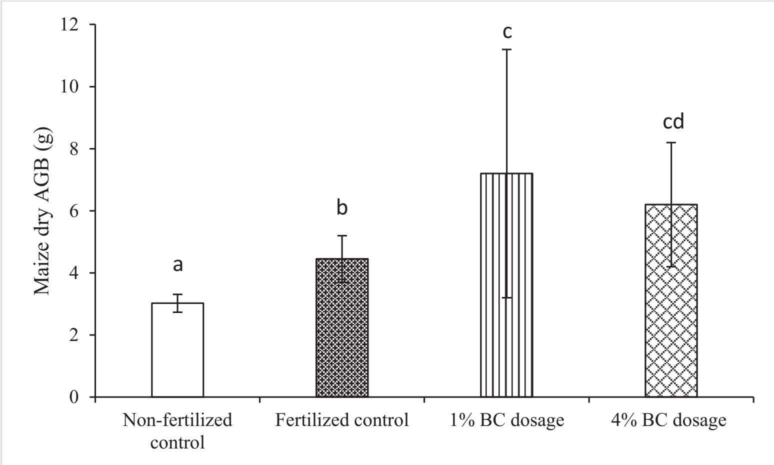

Fig. A: **Effect of 1% biochar dosage (n=43) and 4 % biochar dosages (n=46) and control soils (fertilized control; n=5 and non-fertilized control; n=4) on maize dry AGB (g) yield**. Average maize yield data (1% and 4% biochar dosages) were pooled from biochar produced from different kilns and enriched in various ways. Mean maize AGB yield (g) plotted in y-axis in response to biochar amended (1% and 4% biochar) and control soils on x-axis. Mean AGB yield (g pot^{-1}) along with error bars and their respective significance codes (a, b, c) were provided above each bar based on one way ANOVA and post hoc tukey test at 0.05 significance level.

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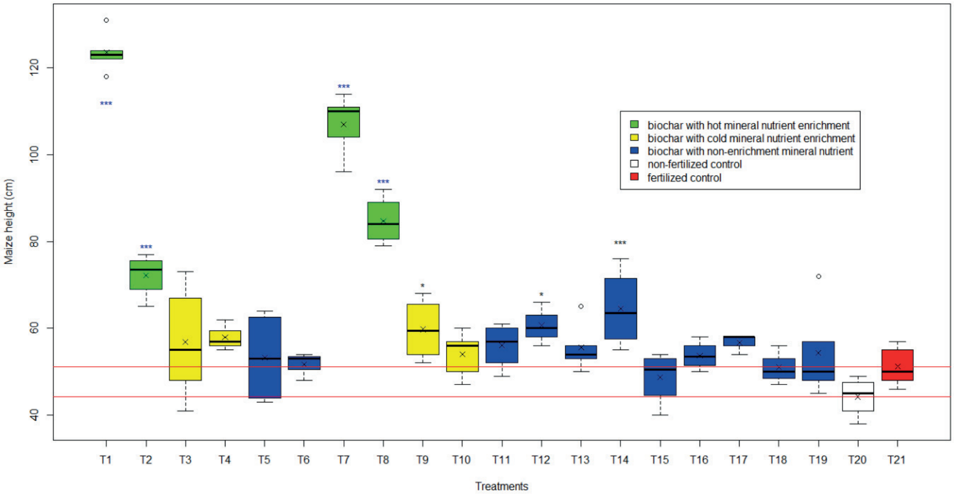

Fig B: Effect of biochar (BC) amended soils produced from different kiln and enriched and non-enriched in various ways with 1 and 4% dosages (19 levels) vs control treatments (2 levels) on maize height. Description of treatments code (T1, T2,…T21) is mentioned in the table 1 and S4. Sign (x) in the middle of the box plot refer to the average maize AGB of each treatments. Asterisk (*) at the top of the box plot denotes the significant difference between biochar treatments over control (C1/T20 for non-fertilized control and C2/T21 for fertilized control) treatments (*** < 0.001, ** <0.01 and * <0.05 significance) based on one way ANOVA (*levels = 21, N=86 and P < 0.0001)* followed by post hoc tukey test (P <0.05). Blue color asterisk (*) represents significance level for both C1/T20 (no color) and C2/T21 (red color box plot) whereas black color (*) only for C1/T20.

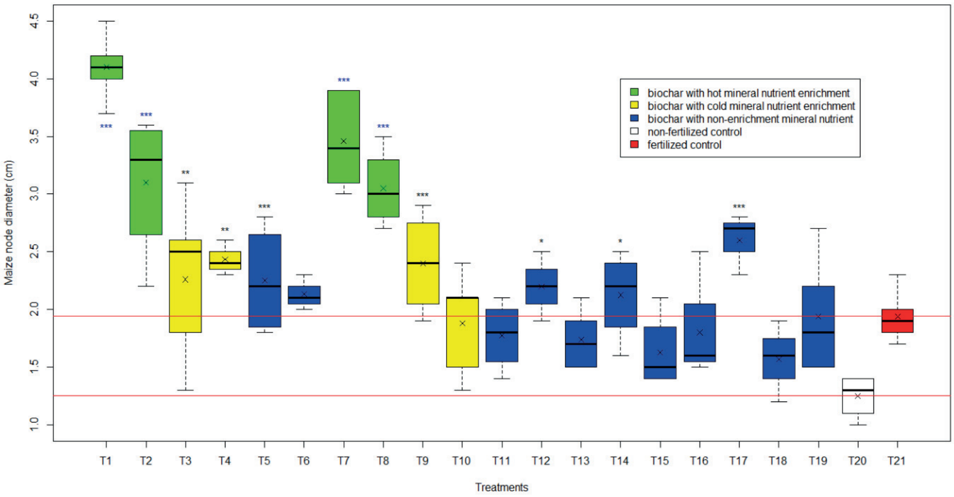

Fig C: Effect of biochar (BC) amended soils produced from different kiln and enriched and non-enriched in various ways with 1 and 4% dosages (19 levels, T1, T2..T19) vs control treatments (2 levels, T20 and T21) on maize node diameter. Description of treatments (T1,T2,…T21) is mentioned in the table 1 and S4. Sign (x) in the middle of the box plot refer to the average maize AGB of each treatments. Asterisk (*) at the top of the box plot denotes the significant difference between biochar treatments over control (C1/T20 for non-fertilized control and C2/T21 for fertilized control) treatments (*** < 0.001, ** <0.01 and * <0.05 significance) based on one way ANOVA (*levels = 21, N=86 and P < 0.0001)* followed by post hoc tukey test (P <0.05). Blue color asterisk represents significance level for both C1/T20 (no color) and C2/T21 (red color box plot) whereas black color (*) only for C1.

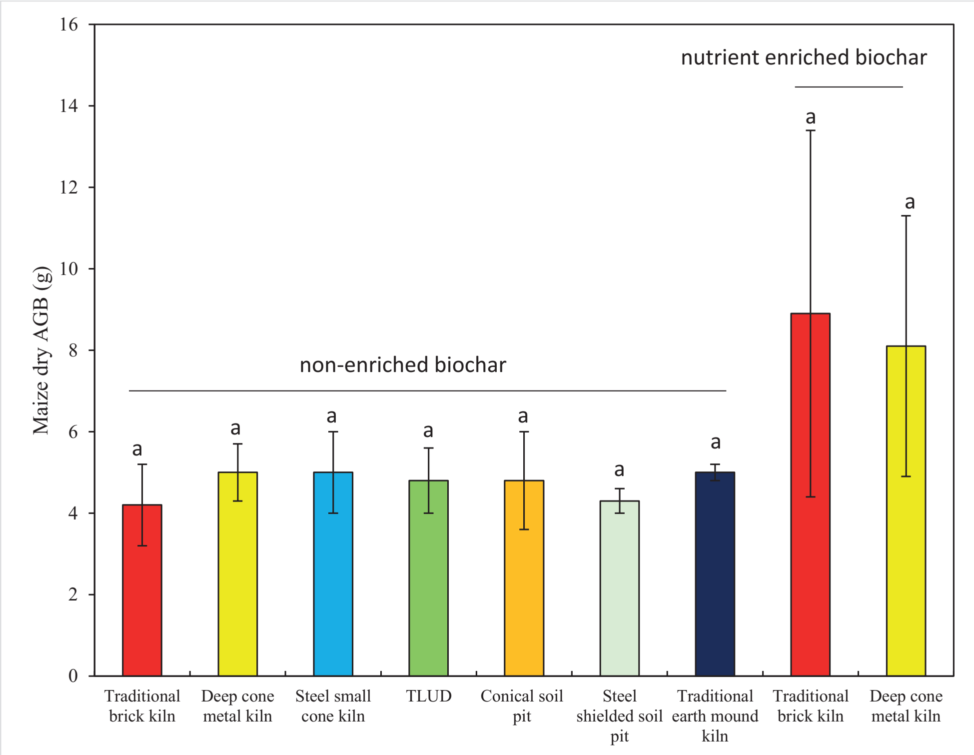

Fig D. Effect of kiln types biochar on maize dry AGB (g) yield. Mean maize AGB yield (g) along with their error bars plotted in y-axis in response to kiln type's biochar for non-enriched (left) and enriched biochar (right) on x-axis. Letters (a) above the bars represents significance level on maize AGB as a function of kiln types biochar following two way ANOVA (N=77).

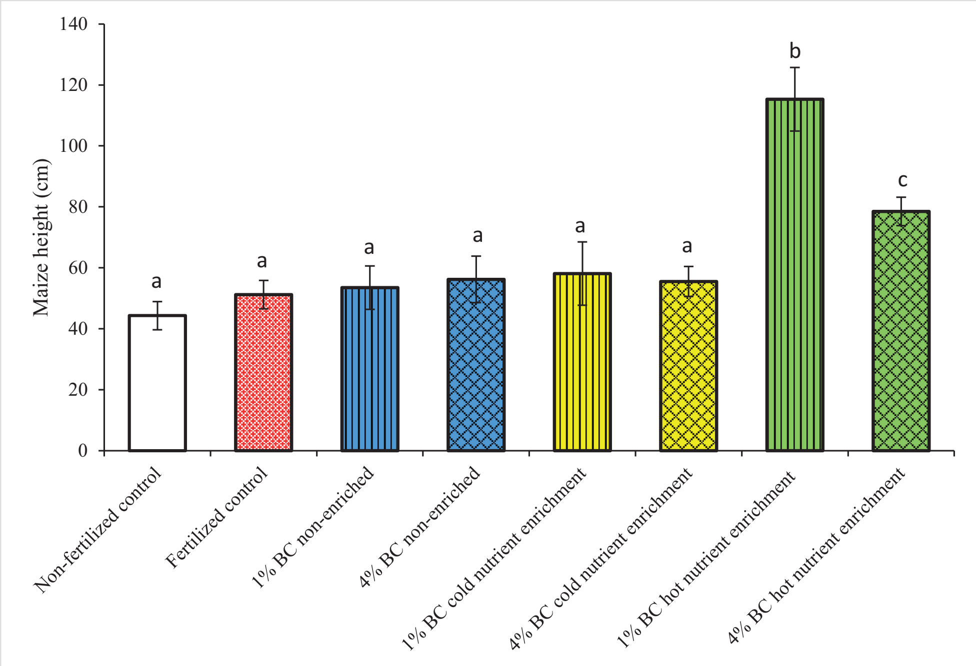

Fig. E. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize height. Maize height (cm) is plotted against y-axis and mineral nutrient enrichment techniques along with controls in x-axis. Different letters above the bars (a, b, c) represent significant differences between mineral nutrient enrichment types following two factor ANOVA (N= 77) and post hoc tukey test at 0.05 significance level.

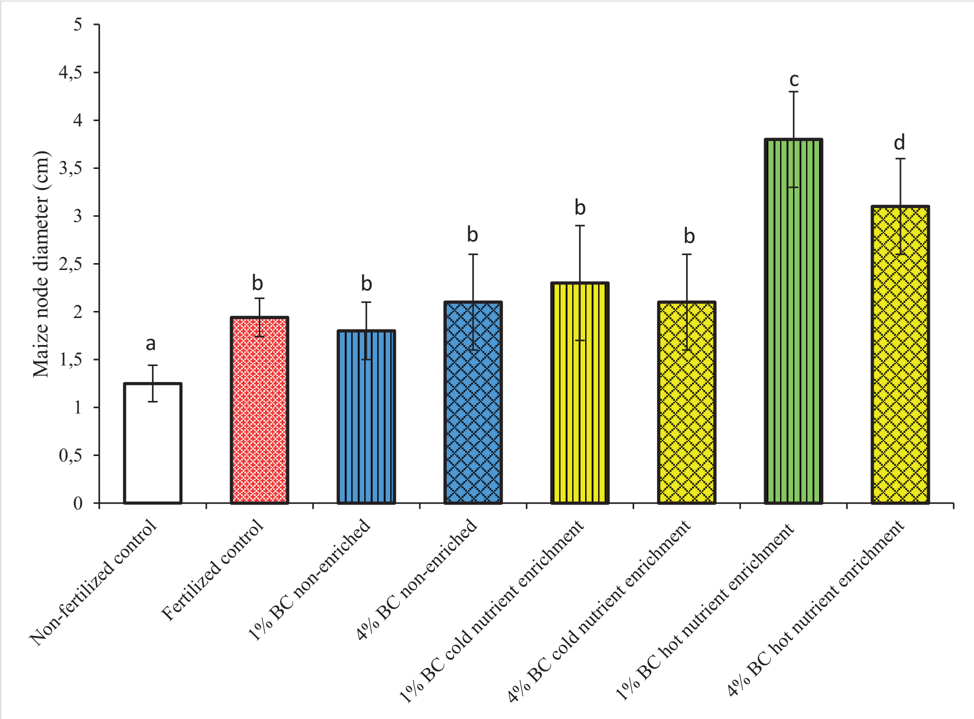

Fig. F. Effect of hot and cold mineral nutrient enrichment and non-enrichment type's biochar on maize node diameter. Maize node diameter (cm) is plotted against y-axis and mineral nutrient enrichment techniques in x-axis. Different letters above the bars (a, b, c) represent significant differences between mineral nutrient enrichment types following two factor ANOVA (N= 77) and post hoc tukey test at 0.05 significance level.

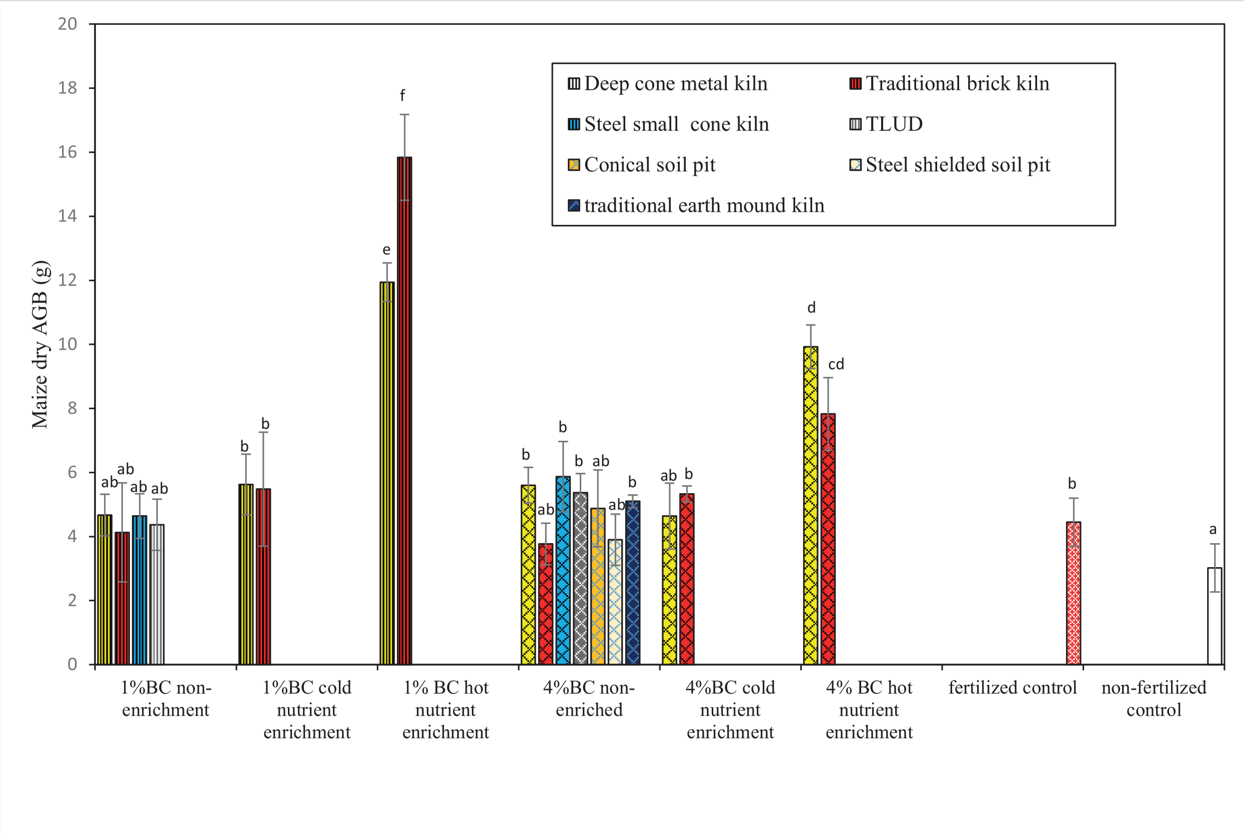

Fig. G. Effect of Kiln type biochar (1% and 4% dosages) enriched and non-enriched with mineral nutrient on maize biomass production. Mean maize AGB yield (g) plotted in y-axis in response to the mineral nutrient enriched and non-enriched biochar produced from different kiln along with controls on x-axis. Different letters above the bars (a, b, c) represent significant differences between the treatments following two factor ANOVA ($N = 77$) and post hoc tukey test ($P < 0.05$).

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Paper III.

Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Hans Peter Schmidt, Gerard Cornelissen

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Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil

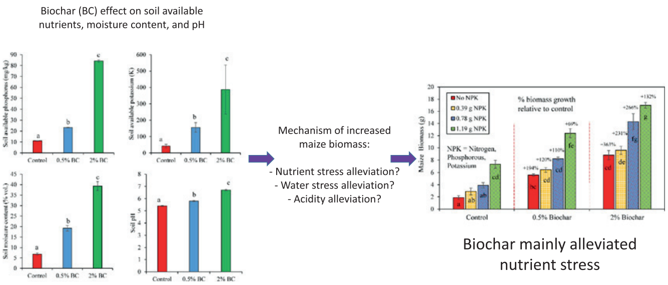
Naba Raj Pandit [a,b,c], Jan Mulder [b], Sarah Elizabeth Hale [a], Vegard Martinsen [b], Hans Peter Schmidt [d], Gerard Cornelissen [a,b,*]

[a] Norwegian Geotechnical Institute (NGI), Oslo, Norway
[b] Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway
[c] Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal
[d] Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, 1974 Arbaz, Switzerland

HIGHLIGHTS

- Soil limitations (moisture, nutrients, acidity) were manipulated one by one to find out why biochar improved crop growth.
- Biochar addition increased soil pH, plant available P, K and soil moisture retention in this weathered Nepalese soil.
- The biochar effect on plant growth was mainly due to alleviation of nutrient stress.

GRAPHICAL ABSTRACT



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ABSTRACT

We studied the role of biochar in improving soil fertility for maize production. The effects of biochar on the alleviation of three potential physical-chemical soil limitations for maize growth were investigated, i.e. water stress, nutrient stress and acid stress. Experiments involved soils with two dosages of biochar (0.5% and 2% w:w), as well as ones without biochar, in combination with four different dosages of NPK fertilizer, water and lime. Biochar was produced from the invasive shrubby weed *Eupatorium adenophorum* using flame curtain kilns. This is the first study to alleviate one by one the water stress, nutrient stress and acid stress in order to investigate the mechanisms of biochar effects on soil fertility.

Biochar addition increased soil moisture, potassium (K) and plant available phosphorous (P-AL), which all showed significant positive relationship ($p < 0.001$) with above ground biomass of maize. However, biochar was much more effective at abundant soil watering (+311% biomass) than at water-starved conditions (+67% biomass), indicating that biochar did increase soil moisture, but that this was not the main reason for the positive biomass growth effects. Biochar addition did have a stronger effect under nutrient-stressed conditions (+363%) than under abundant nutrient application (+132%). Biochar amendment increased soil pH, but liming and pH had no effect on maize dry biomass, so acidity stress alleviation was not the mechanism of biochar effects on soil fertility.

Abbreviations: BC, biochar; CEC, cation exchange capacity; d, days; NPK, nitrogen, phosphorous and potassium; NO_3^--N, nitrate; OC, organic carbon; P-AL, plant available phosphorous; PRS™, plant root simulators; TDR, time domain reflectometer; t ha^{-1}, tonnes per hectare.

* Corresponding author at: Norwegian Geotechnical Institute (NGI), Oslo, Norway.
E-mail address: gco@ngi.no (G. Cornelissen).

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In conclusion, the alleviation of nutrient stress was the probably the main factor contributing to the increased maize biomass production upon biochar addition to this moderately acidic Inceptisol.

1. Introduction

Promising agronomic effects of biochar addition have been found in a wide range of latitude with low-fertile soils (Biederman and Harpole, 2013; Liu et al., 2013), due to improvements of soil biological, physical or chemical properties (Cornelissen et al., 2013; Glaser et al., 2002; Lehmann and Rondon, 2006; Yamato et al., 2006). Biological effects may include enhanced activities of mycorrhizal fungi, ameliorating nutrient uptake by plants (Atkinson et al., 2010) and increased colonization rates of arbuscular mycorrhizal fungi, which for maize plant roots have been shown to increase significantly by 26% for biochar amended soils applied at a rate of 10 l m^{-2} (around 20 t ha^{-1}) (Yamato et al., 2006).

With regard to soil physical properties, biochar addition improved soil water holding capacity (WHC) and plant available water (PAW) in both loamy and sandy loam soils (Bruun et al., 2014; Dugan et al., 2010; Martinsen et al., 2014). WHC was increased by 11% upon biochar amendment (9 t ha^{-1}) in a silty loam agricultural soil, Southern Finland (Karhu et al., 2011). Increased PAW upon biochar addition can be explained by improved porous structure (both microporosity and mesoporosity) and soil aggregation (Herath et al., 2013; Obia et al., 2016). Although it is apparent that biochar can improve soil moisture, there is a knowledge gap regarding to what extent this effect can explain the positive effect of biochar on crop growth.

In addition to soil physical properties, soil chemical properties can also be improved significantly by the addition of biochar. Besides increasing soil pH (higher Ca/Al ratios and higher PO_4^{-3} availability) and base saturation (BS) (Glaser et al., 2002; Martinsen et al., 2015) the addition of biochar increases nutrient retention capacity and soil CEC (Chan et al., 2008; Liang et al., 2006) and thus reduces nutrient leaching (Hale et al., 2013; Laird et al., 2010; Martinsen et al., 2014; Steiner et al., 2007). Low pH is commonly associated with increased Al-concentrations in soil solution, which is highly toxic to plant roots (Gruba and Mulder, 2008). The Al concentration can be reduced drastically by addition of biochar that acts as a liming agent in most of the degraded soils (Glaser et al., 2002; Major et al., 2010; Martinsen et al., 2015; Van Zwieten et al., 2010; Yamato et al., 2006). When 20 t ha^{-1} biochar was applied on highly weathered tropical soils, soil pH increased from 3.9 to 5.1, thereby reducing exchangeable Al^{3+} from 2.67 to 0.12 $cmol_c$ kg^{-1} and exchangeable H^+ from 0.26 to 0.12 $cmol_c$ kg^{-1} whereupon maize yield almost doubled (10 t ha^{-1}) compared with control soils (5 t ha^{-1}) (Yamato et al., 2006). Similar trends were observed for soil CEC, base saturation and exchangeable K upon biochar addition.

Biochar addition can have a strong influence on in-situ soil nutrient availability, emphasizing its role in soil nutrient adsorption and plant availability. Biochar produced from peanut hull at 600 °C showed reduced leaching of NH_4^+-N, NO_3^--N and PO_4^--P (35%, 34% and 21%, respectively) under ex-situ conditions (Yao et al., 2012). The main mechanism may be the absorption of NO_3^--N in biochar nano-pores (Kammann et al., 2015). PO_4^--P is tightly bound in highly weathered tropical soils that are often rich in Fe and Al oxides (Hale et al., 2013). Under such conditions, biochar addition increases soil pH which makes PO_4^--P more bio-available in soil solution (Asai et al., 2009; Hale et al., 2013). For many biochars, an increase in soil K availability may be due to high content of K in biochar ash and reduced K leaching upon biochar addition (Laird et al., 2010; Martinsen et al., 2014).Adding biochar during composting results in organic coatings being formed in the biochar pores (Hagemann et al., 2017), which retains and facilitates slow release of most important plant nutrients (Joseph et al., 2017). Similar to the effect of biochar on soil moisture, it is often unclear to what extent biochar's effect on nutrient retention explains its positive agronomic effects.

Although biochar improves soil quality, there is a knowledge gap regarding the exact mechanism resulting in this positive agronomic effect, as it varies with soil type and the most important soil constraints. Jeffery et al. (2017) conducted a meta-analysis to investigate these soil constraints, and reported 25% average crop yield increase in tropical soils upon biochar addition, mostly through liming and nutrient effects (N and K retention, P availability and direct K addition). However, their study did not allow an individual assessment of the importance of biochar amendment on soil water retention as a factor that could explain the enhanced crop production.

In Nepal, soils are often moderately acidic showing low nitrogen (N), phosphorous (P) and exchangeable base concentrations (Brown et al., 1999; Schreier et al., 1994). Such soil characteristics can have adverse effects on soil fertility and crop production (Brown et al., 1999). Biochar addition has shown positive effects on soil chemical properties in Nepal, with increased pH, CEC and organic C (Pandit et al., 2017) and crop growth (Schmidt et al., 2015). However, thus far, few studies have been published where explicit attempts were made to unravel the soil physical and chemical mechanisms responsible for the positive effect of biochar on crop production. In the present study, the effect of biochar on an acidic silty loam Inceptisol from Rasuwa, Nepal was explored. This soil can be considered representative for many moderately weathered, eroded and acidified soils of Nepal and beyond. Three possible physical-chemical limitations for crop production were anticipated for this soil: i) nutrient limitation due to insufficient nutrient retention ("nutrient stress"), ii) drought due to limited water retention capacity ("water stress"), or iii) a possible degree of aluminum toxicity due to a soil pH_{CaCl2} of 4.5 ("acid stress"). The effectiveness of biochar in alleviating these limitations under maize plantation was evaluated by individual testing of each possible limitation under controlled greenhouse conditions. Controlled conditions were chosen since the main purpose of the present study was mechanistic understanding of biochar effects on soil fertility. Small standard deviations in the biomass data were a prerequisite for the observation of significant differences. Thus, controlled greenhouse conditions were a better choice to execute such a mechanistic study than natural open field conditions. A forthcoming study will report on crop yield data for maize and mustard in exactly the same soil under natural conditions.

We explored the following three hypotheses in the present soil.

1) Biochar alleviates moisture stress through enhanced soil water retention, thus increasing plant growth.
2) Biochar alleviates nutrient stress by increased in-situ nutrient availability (increased CEC) and by the direct addition of nutrients, especially K.
3) Biochar alleviates acid stress and thus increases plant growth by increased soil pH, Ca/Al ratios and available P.

2. Materials and methods

2.1. Soil and biochar

Soil (Inceptisol-Dystrochrept order; IUSS working group WRB, 2006) was collected from agricultural land, Rasuwa district, Nepal (N 28° 00′, E 85° 10′). Soil was collected from 0 to 30 cm depth (top layer) from 20 different locations within a plot (300 m^2) to make a composite soil of approx.600 kg (30 kg each from one location), which was sufficient to carry out this pot trial experiment. The sampled soil used in this experiment was moderately acidic (pH_{CaCl2} 4.5; pH_{water} 5.1), low-CEC (6.05 $cmol_c\ kg^{-1}$ extracted with 1 M NH_4NO_3), silty loam (Supplementary Table 1).

Biochar was produced from ubiquitous, invasive and non-palatable shrubby wood "*Eupatorium adenophorum*" feedstock using a flame curtain steel-shielded soil pit "Kon-Tiki" kiln with final pyrolysis temperature of 600–700 °C. Biochar was finely ground before application (<2 mm).

Details on biochar production technology and properties of produced biochar are shown in Supplementary information files (Supplementary description 2 and Supplementary Table 1).

2.2. Experimental design

A pot trial was carried out under greenhouse conditions (11th May to 5th July 2016) at Matatirtha, Nepal (N 27° 41′ 51″, E 85° 14′ 0″). Average temperature inside the greenhouse was 28.0 ± 8.9 °C (average min. 19.5 °C and average max. 36.5 °C, n = 50) throughout the trial. Pots (top, middle and bottom diameter: 24 cm, 19 cm and 12 cm respectively; height 20 cm; volume 6 l) were filled with 3 kg of air-dried soil-biochar mixtures. Pot size was similar to our previous pot trial study carried out with maize, harvested after 50 d in the same soil (Pandit et al., 2017). Three sets of experiments were set up, each to test the alleviation of one stress factor; i.e. water stress, nutrient stress or acid stress alleviation by biochar. Nutrient stress was created by NPK fertilizer addition at four dosages ranging from very low amounts up to the recommended dosages, water stress was created by watering at four amounts below those provided by normal rainfall (calculation based on average rainfall in the rainy season in Kathmandu, and pot diameter, as in Pandit et al., 2017), and acid stress was alleviated to variable extents by liming (powdered $CaCO_3$) at four dosages to a previously tested range of pH values. Alleviation of the stresses was investigated by adding three different biochar dosages; control (0 t ha^{-1}), 0.5% biochar (10 t ha^{-1}) and 2% biochar (40 t ha^{-1}). Biochar amended at 2% dosage may be a relatively high dosage under farming conditions, but in this study, this dosage was included for mechanistic purpose, and a dosage of 0.5% was also studied for comparison. Under normal growing conditions of maize plants in the field, tillage, weather conditions and other external factors may have an impact on soil properties and crop growth, and this was not considered here. However, we managed to explore the mechanistic study under well-controlled greenhouse conditions excluding all the external abiotic and biotic disturbances that may otherwise hinder the clear illustration of biochar effects in this soil.

Each set of experiments comprised nine treatments with four replications each in completely randomized design (n = 4) resulting in 36 pots per experiment (Table 1). Three treatments receiving the highest amount of water (200 ml per pot per day), NPK (1.17 g per pot) and lime (4.7 g per pot) were added that were considered as shared treatments (common) for each of the water stress, nutrient stress and acid stress sets of experiments. Details of the experimental design and the added amounts of water, NPK and lime for three different dosages of biochar (0% biochar or control, 0.5% biochar and 2% biochar) are summarized in Table 1. After NPK (in the form of Urea, Diammonium Phosphate and Murate of Potash for N, P_2O_5 and K_2O) and lime (applied as pure $CaCO_3$, 99%, Sigma Aldrich, Norway) had been mixed into the soil (air dried not sieved), the pots were left for four days in the green house before maize was planted.

Three maize seeds (*Zea mays;* Manakamana-4 variety) were sown 2 cm below the soil surface in each pot. All the pots from the three sets of experiments, including the water stress alleviation tests, were irrigated daily with 200 ml water per pot and day (corresponding to about 4 mm rainfall per day) until second leaf emergence (14 d). At this point, the smaller and least robust plants were removed, leaving the most robust plant in each pot. After 14 d, all pots were irrigated at five-day intervals with 1000 ml water per pot (20 mm rainfall), except for the pots in the water stress experiment, which received less water (Table 1). The plant water status of maize plants growing in 6 l pots is reasonably representative of that in plants growing in field conditions, since maize root systems in similar soils in the field have been found to constitute only 2–4% of total biomass (root-to-shoot-ratios of 0.02–0.04) and the root system weighed only 10–12 g (Abiven et al., 2015). No crowding of plant roots in the pots was observed. After 30 d, all the pots were top dressed with urea (0.3 g N per pot) except for the nutrient stress experiment which received less N (both basal application and top dressed) than the full dosage (Table 1). Pots were rotated every four days until harvest to ensure the homogeneity of the treatments. Manual weeding was carried out twice (30 d and 42 d) during the experiment.

2.3. In-situ soil measurement

2.3.1. Soil moisture content

Soil moisture content (% by volume) was measured in the water stress alleviation treatments only, using a hand-held Time-Domain Reflectometer (TDR; SM150 soil moisture sensor, Delta T devices Ltd.,

Table 1

Number of treatments in water stress, nutrient stress and acid stress experiment. Each experiment consisted of 9 quadruplicate treatments (n = 4) excluding common or shared treatments (for all three experiments) receiving full water (200 ml per day), NPK (1.17 g per pot) and lime (4.5 g per pot) that was mixed with three different dosages of biochar (0% BC, 0.5% BC and 2% BC) following completely randomized design.

Treatments	Irrigation (ml/pot/day)	NPK basal dose (g per pot)			N top dress (g per pot)	Total NPK (g per pot)	Lime (g per pot)
		N	P_2O_5	K_2O			
Water stress alleviation experiment							
20% water + 0% BC (control), 0.5% BC, 2% BC	40	0.32	0.32	0.23	0.3	1.17	4.5
40% water + 0% BC (control), 0.5% BC, 2% BC	80	0.32	0.32	0.23	0.3	1.17	4.5
70% water + 0% BC (control), 0.5% BC, 2% BC	140	0.32	0.32	0.23	0.3	1.17	4.5
Nutrient stress alleviation experiment							
No NPK + 0% BC (control), 0.5% BC, 2% BC	200	0	0	0	0	0	4.5
1/3rd NPK + 0% BC (control)[a], 0.5% BC, 2% BC[a]	200	0.11	0.11	0.07	0.1	0.39	4.5
2/3rd NPK + 0% BC (control), 0.5% BC, 2% BC	200	0.22	0.22	0.14	0.2	0.78	4.5
Acid stress alleviation experiment							
No lime + 0% BC (control)[a], 0.5% BC[a], 2% BC[a]	200	0.32	0.32	0.23	0.3	1.17	0
0.25 g lime + 0% BC (control), 0.5% BC, 2% BC	200	0.32	0.32	0.23	0.3	1.17	0.25
0.75 g lime + 0% BC (control), 0.5% BC, 2% BC	200	0.32	0.32	0.23	0.3	1.17	0.75
Common or shared treatments							
Full + 0% BC (control)[a], full + 0.5% BC[a], full + 2% BC[a]	200	0.32	0.32	0.23	0.3	1.17	4.5

[a] PRS™ probes treatments selected from all experiment to explore in-situ soil nutrient supply rates.

Burwell, Cambridge, England) just before each irrigation. For each pot and at each time point, three measurements were carried out and averaged to give one reading per pot. Saucers were fitted at the bottom of each pot to measure the amount of water that drained through the pot holes. Importantly, hardly any water drained during the trial from either of the treatment pots.

2.3.2. Soil nutrient availability

Plant root simulator probes (PRS™; Western Ag, Saskatoon, Canada) were used to measure the in-situ availability of cations and anions in the soil (Martinsen et al., 2014). For these measurements, eight treatments were selected; two from nutrient stress, three form acid stress and three from common/shared treatments (Table 1a). Four anion probes and four cation probes per pot were inserted on day 36 (6 days after the addition of the urea top dressing) and left in the soil for 14 days (total 12 + 12 probes per treatment). After exposure, probes were washed thoroughly with water to ensure removal all soil particles. The 12 anion and 12 cation probes per treatment were combined into triplicate anion- and cation-probe samples. PRS™ probes were stored in a cool place after sampling and shipped to Western Ag innovations (Canada) for extraction and analysis according to (Martinsen et al., 2014). Nutrient supply rates measured by PRS ™ probes are reported in μg per 10 cm^2 (sampler surface area) per 14 d (exposure period), i.e., in μg 10 cm^{-2} 14 d^{-1}.

2.3.3. Soil pH

Soil pH was measured at the start (1 d), mid-way (25 d) and end of the experiment (50 d) for soil samples from the acid stress alleviation treatments. Soil pH was measured with WTW pH 320 equipment in 0.01 M $CaCl_2$ solution (1:2.5, solid to solution ratio). Soil pH measured at 1 d, 25 d and 50 d were averaged to give one final reading per treatment.

2.4. Leaf Porometry

Stomatal conductance (mmol water m^{-2} s^{-1}) was measured as an indication of plant water stress for the water stress experiment only (Decagon SC-1 leaf porometer, Seattle, WA, USA). Stomatal conductance was determined from the measured difference in relative humidity between two sensors in the diffusion pathway through a chamber between the leaf surface and a desiccant (Decagon, Seattle, WA, USA) at relative humidity of 0%. Measurements were conducted for 30 s. Calibration was carried out using a wetted Teflon disk with a known conductance of 240 mmol m^{-2} s^{-1}. Measurements were carried out for four different leaves of each plant, giving 16 measurements per quadruplicate treatment. Measurements were carried out on day 50 of the experiment, at a temperature of 20 to 21 °C inside the greenhouse, during continuously rainy conditions at 91 to 92% relative humidity, with very little variation in light conditions during the 4 h of data collection (12 noon to 4 pm). For practical reasons, we could carry out this measurement only once, but under representative conditions for the Nepal rainy season. Measurement accuracy was 10%. During the measurement time interval, 16 background measurements were done without any leaf present in the porometer chamber. Reported values were corrected for the measured background conductance of 26.3 ± 4.7 mmol m^{-2} s^{-1}.

2.5. Plant harvest

Maize plants were harvested on day 50. Maize above ground biomass of all the treatment pots were oven dried at 70 °C for 24 h, prior to measuring dry weight. Roots systems were not considered in this study as the root constituted only a small portion of total biomass (2–4%), and the determination of root biomass is often less accurate than that of biomass because of incomplete soil/root separation and loss of roots during cleaning.

2.6. Ex-situ soil parameters

Triplicate soil samples from each pot (surface layer to 8 cm depth) were collected after harvesting maize plants and pooled into one composite sample for each of the 30 treatments. Soil samples were oven dried at 40 °C for three days and passed through a 2 mm sieve prior to analysis. Particle size distribution of the soil was measured through pipette method. Soil cation exchange capacity (CEC) was measured for the three common treatments (control, 0.5% biochar and 2% biochar which received full amount of water, NPK and lime) to assess the pure effect of biochar addition on soil exchangeable ions. The soil was extracted with 1 M NH_4NO_3 and the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}) were measured in the leachates using inductively coupled plasma optical emission spectrometry (ICP-OES). Exchangeable H^+ was determined by titration with 0.02 M NaOH to pH 7. Sieved samples were crushed for total C, H and N analysis with a CHN analyzer (LECO, Truspec). Plant available phosphorous (P-AL) was measured by the ammonium lactate method (Krogstad et al., 2008), where 40 ml of ammonium lactate solution was added to 2 g dry soil (sieved <2 mm) and shaken in a rotating shaker (1.5 h), and filtered, (0.45 μm). Ascorbic acid (0.4 ml) and molybdenum reagent (0.4 ml) was added to both standard solution and the extracted soil samples and measurements were done using a spectrophotometer (Gilford Stasar Spectrophotometer) at 700 nm. Plant available water (PAW % vol.) was measured for three common treatments (as for CEC above) to explore the effect of biochar addition on water retention capacity. For this purpose, hand-packed soil samples were saturated and soil water measured at different matrix potentials (pF 2, field capacity and pF 4.2, wilting point) through ceramic pressure plates (Martinsen et al., 2014; Obia et al., 2016). PAW (vol%) was calculated as the difference between field capacity (vol%) and wilting point (vol%).

2.7. Statistical analyses

Data were analyzed using R statistical software version 3.2.2. Normality and homogenous variances of all data sets were tested with Shapiro-Wilk- and Levene's test. Two factor ANOVA (fixed effect model) was used for each of the three experiment to assess the effect of the two independent fixed factors (three levels of BC dosage and four levels of either water content, NPK rate or lime rate) including interactions on selected dependent variables. For PRS™ probes datasets one way fixed effect ANOVA model was used to investigate the effect of various treatments comprised of biochar, NPK and lime addition on soil nutrient availability (NO_3^-, PO_4^{3-} and K^+) (dependent variable). Based on our relatively limited PRS™ data availability, one-way ANOVA was chosen. Basically, three factor ANOVA would be the best choice of analysis to show the main effect and interaction effect of biochar, NPK and lime on soil nutrient supply. However, our data could be analyzed only for the main effect of biochar, NPK and lime but not their interaction effect, due to lack of replications or observations for these three factor combinations. Factors showing significant effect were further explored via post hoc Tukey test ($P = 0.05$) to evaluate the significant differences between the treatment means. Analysis of covariance (ANCOVA) was endeavored to see if there is any confounding effect on biomass in each of the three set of experiment such as NPK and pH effect (covariates) under water stress experiment, water and pH effect (covariates) under nutrient stress experiment and water and NPK effect (covariates) on acid stress experiment (described in Supplementary description 1). For this purpose, each of the datasets were pooled based on the biochar effect measured on the soil factors (soil moisture content, pH, and nutrient supply rates) and carried out the ANCOVA model. Pooling the datasets from different set of experiments did not allow the precise explanation of the estimation of various explanatory soil variables on biomass production in the respective pots. In addition, there was hardly any confounding effect observed on each of the three experiments. Therefore, the ANCOVA model was reduced and two-factor ANOVA model was explored for each of the three sets of experiments. Both linear and non-linear regression analysis was included to investigate relationships between selected explanatory continuous independent variables and dependent variables (including biomass) to explain the model. With a view to assess the main effect of biochar addition (control, 0.5% biochar and 2% biochar) on soil physical and chemical properties (dependent variables), one way ANOVA, followed by a Tukey test ($P = 0.05$) were used to explore the significant differences between the biochar and non-biochar treatments (Table 1). The difference between various treatments was significant at $P < 0.05$, unless stated otherwise.

3. Results

3.1. Effect of biochar on soil properties

Biochar addition (2% w:w) significantly increased soil water retention at field capacity (from 29.8 ± 1.8% to 35.3 ± 0.2%) and plant available water (from 20.8 ± 1.9% to 25.5 ± 0.5%) in this soil (Table 2).

Biochar also increased soil CEC and pH as well as exchangeable K^+, Mg^{2+} and Ca^{2+} (Table 2). Biochar addition showed significant effect ($P < 0.001$) on plant available phosphorous (P-AL) which was increased from 11.1 mg kg^{-1} (control) to 23.4 mg kg^{-1} and 84.1 mg kg^{-1} upon 0.5% and 2% biochar addition, respectively (Table 2).

Based on a simple addition of the amount of carbon in the soil and that via the biochar amendment (that is the addition of biochar containing 70% C for 0.5% and 2% biochar dosages to the present soil organic carbon (1.35% SOC)), the resulting soil organic carbon contents should have been 1.70% and 2.75%, close to the observed values of 1.64 and 2.94%, respectively (Table 2).

Table 2
Effect of biochar on soil physical and chemical properties. Treatments with different biochar dosages (0% BC or control, 0.5% and 2% BC) receiving highest amount of agricultural inputs (water, NPK and lime) i.e. the three common treatments. Soil properties values are given as mean ± SD, n = 3. Letters a, b and c denotes significant differences between biochar vs non-biochar (control) treatments on soil properties.

Properties	Common treatments with full NPK, lime and watering rates		
	0% BC (control)	0.5% BC	2% BC
Total organic C%	1.35 ± 0.0 a	1.64 ± 0.01 b	2.94 ± 0.02 c
Total nitrogen%	0.12 ± 0.01 a	0.12 ± 0.01 a	0.14 ± 0.01 a
Total hydrogen%	0.48 ± 0.01 a	0.47 ± 0.01 a	0.48 ± 0.00 a
pH (0.01 M $CaCl_2$)[a]	5.34 ± 0.15 a	5.87 ± 0.13 b	6.58 ± 0.13 c
CEC ($cmol_c$ kg^{-1})	7.63 ± 0.7 a	8.69 ± 0.45 a	11.92 ± 0.24 b
Ca^{2+} ($cmol_c$ kg^{-1})	5.96 ± 0.24 a	6.38 ± 0.24 a	8.87 ± 0.24 b
Mg^{2+} ($cmol_c$ kg^{-1})	0.54 ± 0.02 a	0.67 ± 0.01 b	1.07 ± 0.04 c
K^+ ($cmol_c$ kg^{-1})	0.26 ± 0.02 a	0.55 ± 0.07 b	1.75 ± 0.12 c
Al^{3+} ($cmol_c$ kg^{-1})	0.03 ± 0.03 a	0.006 ± 0.00 a	0.006 ± 0.00 a
H^+ ($cmol_c$ kg^{-1})	0.81 ± 0.84 ab	1.05 ± 0.19 a	0.17 ± 0.14 b
Sand %	32.70 ± 0.49	32.1 ± 0.35	32.70 ± 0.49
Silt %	49.90 ± 0.43	50.6 ± 0.55	50.70 ± 1.05
Clay %	17.40 ± 0.11	17.40 ± 0.37	16.70 ± 0.60
Textural class	Silty loam	Silty loam	Silty loam
Soil moisture content (% vol.)[b]	6.9 ± 0.6 a	19.1 ± 1.4 b	39.3 ± 2.1 c
Field capacity (% vol)	29.83 ± 1.83 a	29.96 ± 1.34 a	35.30 ± 0.18 b
Plant available water (% vol)	20.82 ± 1.97 a	21.18 ± 0.78 a	25.55 ± 0.54 b
P-AL (mg kg^{-1})	11.10 ± 0.30 a	23.36 ± 0.28 b	84.16 ± 1.08 c
PRS™ adsorbed cations and anions			
NO_3^{-1} (μg per 10 cm^2)	304 ± 158 a	636 ± 131 a	783 ± 257 a
Ca^{2+} (μg per 10 cm^2)	1350 ± 386 a	2401 ± 645 b	2259 ± 99 b
Mg^{2+} (μg per 10 cm^2)	103 ± 45 a	223 ± 18 b	284 ± 30 b
K^+ (μg per 10 cm^2)	41 ± 11 a	156 ± 29 b	384 ± 144 c
P (μg per 10 cm^2)	1.2 ± 0.4 a	3.1 ± 0.4 b	3.5 ± 3.3 b
Fe^{3+} (μg per 10 cm^2)	40 ± 23.7 a	103 ± 4 b	86 ± 27 b
Al^{3+} (μg per 10 cm^2)	31 ± 16.6 a	54 ± 16.8 a	24 ± 6.7 a
Ca/Al (molar ratio)	32.2 ± 9.0 a	32.3 ± 17.7 a	63.8 ± 18.6 b

[a] Soil pH was averaged and pooled for standard deviation from 1 d, 24 d and 50 d (in-situ and ex-situ pH measurement) to give one final reading (mean ± SD).
[b] Daily measured in-situ soil moisture percentage measurement (% vol.), n = 50.

3.2. Alleviation of water stress by biochar

3.2.1. Effect of biochar on soil moisture content

Soil moisture percentage increased up to seven-fold upon 2% biochar addition for both highest watering (200 ml per day, increased moisture content from 7% to 40% by vol.) and lowest watering rates (40 ml per day, increased moisture content from 1% to 7%) (Fig. 1, Supplementary Fig. 1).

3.2.2. Effect of biochar on maize biomass and stomatal conductance at various watering rates

A significant interaction between the effect of biochar dosages and watering rates on maize biomass production was observed (Fig. 2a, Table 3a-ii). Biochar addition showed significant effects on biomass at all watering rates (the presence of 2% biochar increased biomass by +67 to +311% dependent on watering rate; Fig. 2a), but slightly less so at the lowest water addition (40 ml per day and 80 ml per day), where only the 2% biochar dosage but not the 0.5% dosage showed significant increments on biomass production (Fig. 2a). Leaf stomatal conductance showed a positive relationship ($R^2 = 0.37$, $P = 0.03$) with soil moisture content (Supplementary Fig. 2a) and dry biomass production ($R^2 = 0.51$, $P = 0.008$) (Supplementary Fig. 2b).

3.3. Biochar and nutrient stress alleviation

3.3.1. Effect of biochar on soil nutrient availability

PRS™ probe measured K^+ and PO_4^{3-}-P rates (all in units μg 10 cm^{-2} 1 d^{-1}) were significantly higher upon biochar addition (2% biochar) for both the lowest (0.39 g NPK) and the highest amount of NPK addition (1.19 g NPK) (Table 4). At the lowest NPK rate, biochar addition strongly increased K^+ supply rates from 23.6 ± 2.5 to 667 ± 215 and PO_4^{3-}-P from 1.6 ± 0.6 to 5.5 ± 2.0 (Table 4). Other fertilizer nutrient supply rates such as NO_3^--N, Ca and Mg showed significant effects only upon NPK addition but not on biochar amendment (Table 4). Furthermore, P-AL (mg kg^{-1}) was significantly increased upon mineral nutrient (NPK) addition but the response was stronger when biochar was added (Supplementary Table 2). P-AL (mg kg^{-1}) increased approximately eight-fold in the presence of 2% biochar at all level of NPK additions (Supplementary Table 2).

3.3.2. Effect of biochar on maize biomass at various NPK dosages

A significant interaction between the effect of biochar dosages and NPK rates ($P < 0.001$) on maize dry biomass (Fig. 2b, Table 3b-ii) was observed. Both dosages of biochar increased biomass production at all levels of NPK application (Fig. 2b). The most important trends observed between various PRS™ probes soil nutrient supply rates (Supplementary Fig. 4) and maize biomass production were those for K^+ ($R^2 = 0.51$, $P < 0.001$; Supplementary Fig. 4d) and P-AL ($R^2 = 0.61$, $P < 0.001$; Supplementary Fig. 5.b).

A combination of biochar and low NPK (1/3rd NPK) revealed significantly higher biomass production compared with control (2.9 ± 1.1 g per pot); increases were +120% at 0.5% biochar (6.4 ± 0.7 g per pot) and +231% at 2% biochar (9.6 ± 1.3 g per pot) (Fig. 2b). A similar trend was observed for the combination of biochar and both the second and third NPK addition (Fig. 2b). For the highest NPK rate, biochar addition was observed to have additional but not as strong effects on biomass production (increased by +69% at 0.5% biochar and by +132% at 2% biochar compared with control) (Fig. 2b).

3.4. Biochar and acid stress alleviation

3.4.1. Effect of biochar on soil pH and plant available phosphorous

Both biochar and lime addition showed significant effects ($P < 0.001$) on average soil pH (Fig. 3a, Table 3c-i) measured at 1 d, 24 d and 50 d during the experiment (Supplementary Table 3). A similar trend was observed for the ratio between PRS™ probes extractable Ca and Al

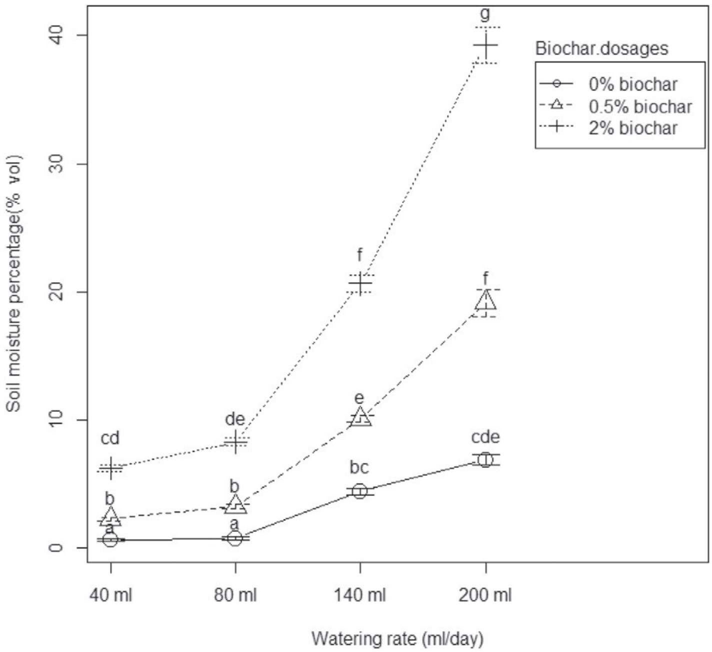

Fig. 1. Effect of biochar dosages and watering rate on soil moisture content (percentage by volume). Soil moisture percentages measured at five-day intervals after second leaf emergence at 15 d until harvest at 50 d. Each level of biochar dose combined with each level of watering rates; mean ± SE, n = 28. Different letters inside the graph denote significant differences between the treatments followed by two factor ANOVA (Post hoc Tukey test, $P < 0.05$).

supply rates (extractable Ca/Al) (Table 4). Biochar addition (2% biochar) significantly increased Ca/Al ratio both in the absence (from 11.3 ± 0.9 to 23.7 ± 7.2) and presence of lime (from 32.2 ± 9.0 to 63.8 ± 18.6) (Table 4). In addition to improved pH and Ca/Al ratio, plant available phosphorous (P-AL) was also significantly increased upon biochar addition, probably as a result of a more favorable pH; increases in P-AL were observed from 11.0 ± 0.3 mg kg^{-1} (control) to 23.3 ± 0.2 mg kg^{-1} at 0.5% biochar and to 84.1 ± 2.0 mg kg^{-1} at 2% biochar addition for the treatments receiving full amount of liming rates (Supplementary Table 2). Improved soil pH illustrated higher plant available phosphorus ($R^2 = 0.75$, $P < 0.01$) attributed mainly by biochar amendment in this soil (Supplementary Fig. 6a).

The Al^{3+} data in Table 4 are very low compared to those of Ca^{2+}, and insignificant compared to the fluxes of base nutrients. The limed treatments also received full NPK, and NPK mineral fertilizer is acidifying. This is probably the reason that Al^{3+}, while still low, was slightly higher in the presence of full NPK (and lime; Table 4).

3.4.2. Effect of biochar on biomass production under various liming rates

Lime addition increased soil pH (Fig. 3a) and Ca/Al ratio in the PRS™ probes membranes (Table 4). However, importantly liming had no effect ($P > 0.05$) on maize biomass production (Fig. 3b, Table 3c-ii). Biochar addition was the only main factor increasing maize biomass production with respect to different liming rates in this experiment (Fig. 3b). Liming and biochar addition did increase P-AL ($R^2 = 0.63$, $P < 0.001$; Supplementary Fig. 6b) but not maize biomass.

4. Discussion

Biochar addition clearly resulted in improved soil moisture content (Fig. 1). Also, maize biomass increased with daily watering rate. However, biochar addition (2% w:w) was less effective under water-stressed conditions (+67% biomass at 40 ml water per day) than in the presence of ample water (+311% at 140 ml water per day). These observations indicate that the biochar, despite increasing soil moisture (Fig. 1), increased biomass yield in ways related to factors other than water stress alleviation. In this respect our data are similar to those of Wang et al. (2016) where biochar addition improved soil moisture but not crop growth. The most important effect of biochar in our soil was most likely nutrient stress alleviation, as biochar showed the strongest effect at the lowest fertilization rates (1/3rd NPK), with the combination of biochar and mineral fertilizer NPK showing a significant and positive effect on biomass production (Fig. 2b, Table 3b-ii).

With regard to alleviation of soil acidity, the effect of biochar on biomass production was much stronger than the effect of liming (Fig. 3b). Indeed, lime addition did not show a significant effect on biomass production (Fig. 3b, Table 3c-ii). Thus, soil acidity (pH of 4.5 in CaCl2 and 5.1 in water and reasonably low exchangeable Al^{3+} of 1.6 $cmol_c\ kg^{-1}$) was not a limiting factor for crop production in this soil. An indirect effect of improved soil pH is often an increase in P-AL in the presence of biochar, so that does not seem to be the mechanism of the biochar effect on biomass. However, biochar did result in a nutrient retention effect, and a positive relationship between P-AL and biomass was observed, so it is well possible that P-AL was improved by biochar in other ways than indirectly via increasing soil pH.

Thus, hypotheses 1 and 3 were falsified with respect to water stress and acid stress respectively, whereas hypothesis 2 was not falsified by the experimental data.

In this study, we could assume the amount of water added to the pots to be constant for all the treatments, as there was no water drained during the trial for either of the biochar and non-biochar treatments. Thus, water loss from the system was mainly governed by soil surface evaporation and plant evapotranspiration. The larger amounts of soil moisture in the 2% BC treatment indicate that there was less water loss here compared to non-amended soil, despite the larger biomass and resulting larger evapotranspiration. Thus, BC probably increased

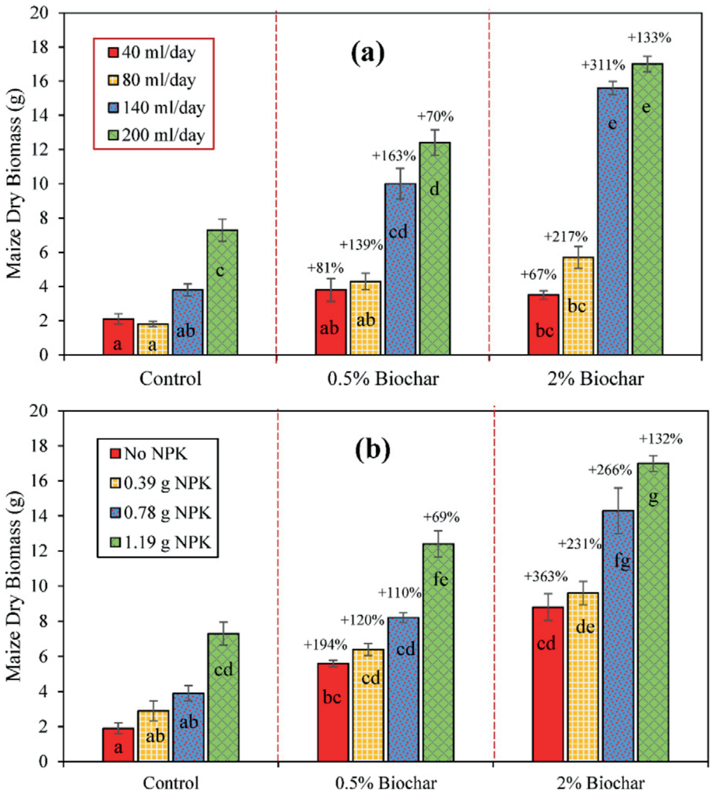

Fig. 2. Dry weight of maize above ground biomass at harvest under water stress experiment (Fig. a) and nutrient stress experiment (Fig. b); mean ± SE, n = 4. Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, $P < 0.05$). The percentage values above the bars denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar, at different watering (Fig. a) and NPK rates (Fig. b).

the water use efficiency (WUE), in accordance with earlier observations (Uzoma et al., 2011), and reduced evaporation from the soil surface, which was previously observed for biochar addition (3% w:w) (Basso et al., 2013). Biochar has recently been shown to form organic pore coatings that improve water retention (Hagemann et al., 2017), by reducing pore space (lowering capillary rise), and boosting hydrophilicity.

Increased PAW upon 2% biochar addition (from 21% to 26%) (Table 2), was in line with data reported by Obia et al. (2016) where biochar addition (0.2 and 4% w:w) increased PAW by 3% in Mkushi loamy soils (maize field), Zambia. Similar trend (an increase of PAW from 18.2% to 22.3%) was reported by Martinsen et al. (2014) upon biochar addition (10% vol.) in the same soil. Even stronger increases in PAW (by ~19%) have been reported for 10 t ha^{-1} biochar application on a silty loam soil of Hawera, New Zealand (Herath et al., 2013).

Stomatal conductance was on the lower end of the range previously observed for maize <100 mmol m^{-2} s^{-1} under drought conditions and 100–200 mmol m^{-2} s^{-1} under fully irrigated conditions (Medici et al., 2007), with the same trend of lower stomatal conductance under water stress (Supplementary Fig. 4).

Table 3
Statistical analysis (two factor fixed effect ANOVA model) under water stress, nutrient stress and acid stress experiments.

Factor	Response variable, *P* value		
a. Water stress experiment	i) Soil moisture content (% vol.)	ii) Maize dry biomass (g)	iii) Stomatal conductance (mmol m^{-2} s^{-1})
Biochar dosages	<0.001	<0.001	0.04
Water rates	<0.001	<0.001	<0.001
Biochar dosages X water rates	<0.001	<0.001	0.14
b. Nutrient stress experiment	i) P-AL (mg kg^{-1})	ii) Maize dry biomass (g)	
Biochar dosages (categorical)	<0.001	<0.001	
NPK rates (categorical)	<0.001	<0.001	
Biochar dosages X NPK rates	0.02	<0.001	
c. Acid stress experiment	i) Soil pH content	ii) Maize dry biomass (g)	iii) P-AL (mg kg^{-1})
Biochar dosages	<0.001	<0.001	<0.001
Lime rate	<0.001	0.8	<0.001
Biochar dosages X lime rate	0.21	0.2	<0.001

Table 4
Cations and anions concentrations adsorbed in PRS™ probes (μg 10 cm^{-2} 14 d^{-1}); mean ± SD, n = 3. Average PRS™ probes adsorbed nutrients (cations and anions) were analyzed through one way ANOVA (*levels* = 8, n = 3, *N* = 24) with subsequent post hoc Tukey test (*P* = 0.05). Different letters inside the parenthesis indicates significant differences (*P* < 0.05) between the various treatments (independent variable) on the adsorbed nutrient parameters illustrated in each column (response dependent variable). NH_4^+ supply rates not shown in the Table as these were very low.

Treatments	NO_3^-	Ca^{2+}	Al^{3+}	Mg^{2+}	K^+	PO_4^{3-}	Ca/Al[a]
0% BC + 1/3 NPK + lime	96.0 ± 51.0 (a)	703.0 ± 114.0 (a)	9.4 ± 1.2 (a)	51.6 ± 0.5 (a)	23.6 ± 2.5 (a)	1.6 ± 0.6 (a)	49.8 ± 1.8 (c)
2% BC + 1/3 NPK + lime	80.6 ± 27.3 (a)	667.6 ± 320.0 (a)	7.0 ± 1.8 (a)	55.3 ± 14.4 (a)	667.6 ± 215.0 (c)	5.5 ± 2.0 (b)	62.8 ± 19.3 (c)
0% BC + full NPK + lime	304.6 ± 158.2 (b)	1350.3 ± 386.0 (a)	30.5 ± 16.5 (ab)	103.0 ± 44.5 (ab)	41.6 ± 10.9 (a)	1.2 ± 0.4 (a)	32.2 ± 9.0 (b)
0.5% BC + full NPK + lime	636.0 ± 131.5 (bc)	2401.0 ± 644.8 (b)	54.5 ± 16.8 (bc)	223.5 ± 17.6 (bc)	155.5 ± 28.9 (b)	3.1 ± 0.3 (b)	32.3 ± 17.7 (bc)
2% BC + full NPK + lime	783.0 ± 257.7 (bc)	2259.0 ± 99.5 (b)	25.0 ± 6.7 (b)	283.3 ± 29.8 (c)	387.3 ± 154.2 (bc)	3.5 ± 3.2 (ab)	63.8 ± 18.6 (c)
0% BC + full NPK + no lime	700 ± 251.4 (bc)	882.6 ± 135.0 (a)	52.0 ± 5.0 (bc)	154.0 ± 30.3 (b)	113.0 ± 31.0 (b)	1.6 ± 0.5 (a)	11.3 ± 0.9 (a)
0.5% BC + full NPK + no lime	620.6 ± 144.8 (bc)	944.0 ± 297.3 (a)	62.7 ± 11.2 (c)	171.3 ± 67.3 (b)	329.0 ± 21.1 (c)	2.7 ± 0.1 (b)	9.8 ± 1.4 (a)
2% BC + full NPK + no lime	344.0 ± 129.6 (b)	625.6 ± 166.3 (a)	20.5 ± 13.5 (ab)	121.3 ± 37.8 (ab)	1002.0 ± 56.6 (d)	5.0 ± 2.1 (b)	23.7 ± 7.2 (b)

[a] Presented in molar ratio.

Biochar changes the soil surface albedo (Verheijen et al., 2013), which may result in an increasing variability in soil moisture. However, in controlled greenhouse conditions with less intense lighting conditions, this effect may be missed (Zhang et al., 2013), somewhat decreasing study relevance but increasing the possibilities to study the direct effects of changes in soil chemistry and soil moisture on plant growth.

Increased K^+ and P-AL supply upon biochar addition, through the 22% ash fraction of the biochar, were probably the main nutrient factors responsible for increased biomass production in this soil. A significant positive relationship between maize biomass production and K supply rates (Supplementary Fig. 4d), combined with previous observations that K is the main nutrient added by the addition of biochar (Martinsen et al., 2014), indicated that the K addition via biochar contributed to the alleviation of nutrient stress by biochar. A recent study by Gautam et al. (2017) reported increased K^+ availability upon biochar addition (5 t ha^{-1}) in silty loam Nepalese soil, the main mechanism being high content of K in biochar ash as well as reduced K leaching (Laird et al., 2010). A similar positive trend was observed between P-AL and biomass production (Supplementary Figs. 5, 6), probably due to increased P-AL, where biochar addition increased P-AL from 6 mg kg^{-1} up to a level of 70 mg kg^{-1} (Supplementary Table 2), within the range of 50–70 mg kg^{-1} required for optimal crop growth (Krogstad

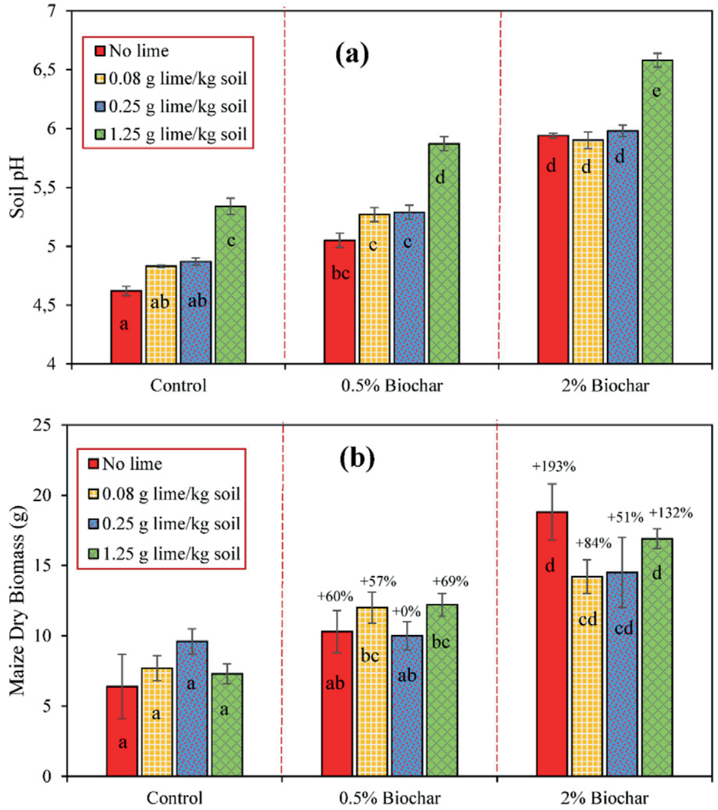

Fig. 3. Effect of the combination of biochar dosages and liming rates on average soil pH (Fig a; mean ± SE, n = 11) and maize biomass production (Fig b; mean ± SE, n = 4). Different letters inside a bar of each treatment represents significant differences between various treatments following two way ANOVA (post hoc-Tukey test, *P* < 0.05). The percentage values above the bars (Fig b) denote the relative change in dry biomass production in the presence of biochar, as compared to the control receiving no biochar.

et al., 2008). Increased P-AL in P-poor soils was reported upon biochar addition, resulting in crop production improvements (Asai et al., 2009).

Nutrient use efficiencies (NUE) were improved by biochar addition at all nutrient dosages; NUE for N was 10–15% without biochar and 30–45% with biochar (assuming the same N content of maize biomass (Martinsen et al., 2014) in the presence and absence of biochar). NUEs for P and K were 6–9% and 21–31%, respectively, in the absence of biochar, and 18–27% and 60–90%, respectively, in the presence of biochar.

A recent study from Jeffery et al. (2017) showed that biochar addition increased crop yield significantly in low fertility soils, highlighting the role of biochar in nutrient stress alleviation. However, in our study, biochar was still effective in the absence of nutrient stress, at highest NPK (132% increase) (Fig. 2b). Thus, the biochar addition in combination with NPK rate had supplementary effects on maize biomass in addition to nutrient retention/addition - probably due to other improved soil physicochemical or biological parameters.

This is the first mechanistic study to investigate the effect of biochar in alleviating some of the most important physical-chemical soil constraints (water stress, nutrient stress and acid stress) by studying the parameters one by one under controlled conditions, under maize plantation in a moderately acidic silty loam Nepalese soil.

In addition to soil moisture and nutrient availability improvements, biological properties of the soil can also be improved by biochar addition. We cannot exclude that beneficial biochar effects on soil (micro) biology, including effects on mycorrhizae, may have contributed to the observed agronomic effects. As the experiments were conducted under controlled greenhouse conditions, any effects related to the effect of biochar on pest resistance could probably be ruled out.

5. Conclusion and recommendations

Soil physicochemical properties such as soil moisture percentage, PAW, in-situ soil nutrient supply rates (PO_4^{3-}, K^+, Ca^{2+}), P-AL, soil pH and CEC were significantly improved upon biochar addition. Increased nutrient availability (K and P-AL) upon biochar addition showed beneficial effect on maize biomass production in this study, thus, alleviating nutrient stress in silty loam soil of Rasuwa, Nepal. The experiment was performed for one soil representative of low-fertility soils. However, maize is more sensitive to drought and nutrient conditions and quite tolerant to low pH conditions than other crops, thus, the results found for maize plant might not be fully representative for other plants. Repetition of the experimental design is recommended for various soils with various limiting factors for crop growth, as well as for various biochar and crop types. In addition, mechanistic field trials similar to the ones carried out in this greenhouse study are recommended.

Farmers can produce biochar themselves at low cost and labour from *Eupatorium* shrub using flame curtain pyrolysis kilns (Schmidt et al., 2015). This pest can be turned into a resource by making biochar to improve soil fertility. This will be of practical importance to identify the potential role of biochar towards sustainable, nutrient efficient agriculture, under rain-fed conditions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.01.022.

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Supplementary Information (SI)

Biochar improves maize growth by alleviation of nutrient stress in a moderately acidic low-input Nepalese soil

Naba Raj Pandit[1,2,3], Jan Mulder[2] , Sarah Elizabeth Hale[1], Vegard Martinsen[2], Hans Peter Schmidt[4], Gerard Cornelissen[1,2]

1) Norwegian Geotechnical Institute (NGI), Oslo, Norway

2) Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway

3) Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal

4) Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, 1974 Arbaz, Switzerland

Supplementary Description 1. Statistical analysis for the prediction of AGB production

Analysis of covariance (ANCOVA) model (full model to adequate reduced model) was used prior to two factor fixed effect ANOVA model to assess the effect of biochar on maize above ground biomass, in general, keeping water%, soil pH, nutrients (K, P-AL, NO_3^-) as independent explanatory continuous variable (covariates) and maize biomass as dependent response variable under three sets of experiment. For instance, under water stress experiment, biochar dosages and watering rates were used as a categorical variable and soil pH and soil nutrient supply (K, P-AL, NO_3^-) as an independent continuous confounding variable on dependent maize biomass. Similarly, under nutrient stress experiment, biochar dosages and NPK rates were used as a categorical variable including soil moisture content and soil pH as an independent confounding variable. Likewise, under acid stress experiment, biochar dosages and lime rates were used as categorical variable and soil moisture content and nutrient supply were used as an independent continuous confounding variable. In this experiment, we don't have complete datasets for each set of experiment. For instance, soil moisture content was measured only from water stress experiment, soil pH only from acid stress experiment and nutrient supply rates only from selected PRS^{TM} treatments. Therefore, under water stress experiment, soil pH and nutrient supply rates values were taken from acid stress and nutrient experiment respectively; for nutrient stress experiment, soil moisture content (%) and pH data were taken from water stress and acid stress experiment respectively, and under acid stress exepriment, soil moisture content and nutrient supply datasets were taken from water stress and nutrient stress experiment respectively to predict the maize biomass under each set of experiment. Each of the datasets were pooled based on the biochar effect measured on the soil factors (soil moisture content, pH, and nutrient supply rates) and carried out the ANCOVA model. Pooling the datasets from different set of experiments did not allow the precise explanation of the estimation of various explanatory soil factors on maize biomass production. Thus, we decided to run two factor fixed effect ANOVA model to assess the effect of biochar on maize biomass for each set of three experiments. For each set of experiment, simple linear regression was carried out to explain the model. For instance, under water stress experiment, biochar addition showed significant positive effect on soil moisture content and maize biomass, and under such situation, we plotted the relationship between soil moisture content and maize biomass to explain the model (effect of biochar on maize biomass). Similar analysis was operated wherever necessary for other set of experiments.

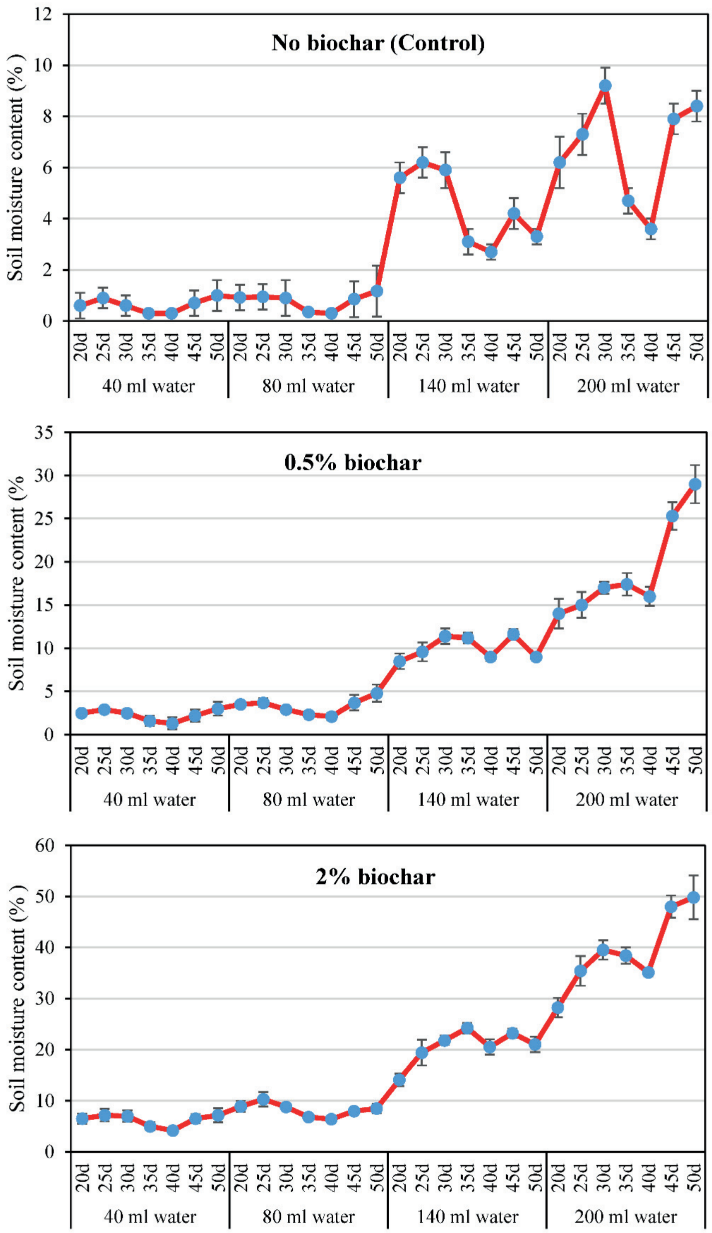
Supplementary Description 2. Biochar production technology

The invasive forest shrub "*Eupatorium adenophorum*" which is about 1-2 m high with stems up to 2 cm in diameter, regenerates naturally and is ubiquitous in forests and river banks throughout Nepal (Pandit et al. 2017; Shrestha et al. 2009). It has been used as a sustainable feedstock to produce biochar (Cornelissen et al. 2016; Schmidt et al. 2015). *Eupatorium adenophorum* with local name "Banmara" (forest killer) is an invading species causing rapid destruction of forest and biodiversity, which has shown negative impact on livelihood sustainability, food security, environment and ecosystem management (Kunwar 2003). Such an invasive forest shrub could be sustainably utilized to produce a valuable biochar thereby relieving the environmental problem. Eupatorium is a novel biochar feedstock in a global perspective, potentially turning a pest into a resource. Thus far, biochar produced from "*Eupatorium adenophorum*" has shown good quality, qualifying for the premium quality of European biochar certificate and boosting agronomic performance when applied in silty loam Nepalese soil (Schmidt et al. 2015). Elemental analysis of *Eupatorium* feedstock (dry weight basis) showed 42.9% C, 1.4% H, 1.5% N (Supplementary Table 1).

Biochar was produced using a flame curtain steel-shielded soil pit "Kon-Tiki" kiln with final pyrolysis temperature of 600-700°C for 2 hour in each run (Cornelissen et al. 2016). Quenching was done by placing an isolating soil layer on top of the kiln (Cornelissen et al. 2016). Biochar was harvested after 24 hour and the top soil quenched layer was thrown away carefully with the help of spade in such a way that the biochar below the layer was not much intermixed with soil. Some biochar particles mixed with soil were handpicked carefully and thrown away. Biochar surface coated with soil layer was excluded and only isolated pure biochar was used under greenhouse experiment. Biochar was crushed and sieved (< 2mm) and mixed thoroughly to ensure its homogeneity before it was mixed into the soil of the different treatment pots. The biochar had a pH_{CaCl2} of 8.9, CEC of 121 $cmol_c\ kg^{-1}$ (1M NH_4NO_3 - extractable base cations), 70.4 % of organic carbon and 74.6 $m^2\ g^{-1}$ of surface area (Table 1). pH, CEC and total CHN % of biochar samples were analyzed in the same way as performed for soil samples. Surface area ($m^2\ g^{-1}$) of biochar was analyzed by N_2 adsorption at 77 K using an automated surface analyzer following similar procedure as mentioned in (Pandit et al. 2017).

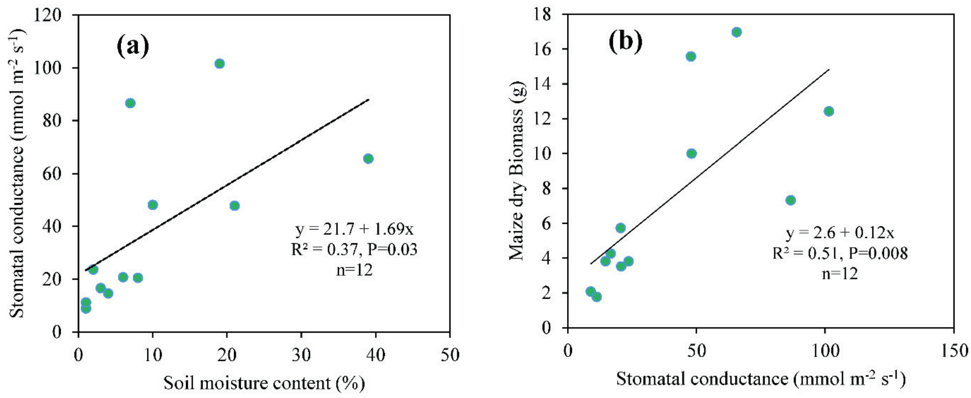
Supplementary Table 1. Chemical properties of feedstock, biochar and soil used in the pot trial experiment

Properties	Feedstock (*Eupatorium*)	Biochar	Soil
Total Carbon %	42.9	70.4	1.35
Total Nitrogen %	1.5	2.16	0.12
Total Hydrogen %	1.4	0.66	0.48
Ash (%; 550 °C)	-	21.9	-
pH (0.01M $CaCl_2$)	-	8.9	4.5
pH (water)	-	-	5.12
CEC ($cmol_c\ kg^{-1}$)	-	121	6.05
Exchangeable Ca^{2+} ($cmol_c\ kg^{-1}$)	-	-	2.3
Exchangeable Mg^{2+} ($cmol_c\ kg^{-1}$)	-	-	0.56
Exchangeable K^{+} ($cmol_c\ kg^{-1}$)	-	-	0.25
Exchangeable Al^{3+} ($cmol_c\ kg^{-1}$)	-	-	1.6
Exchangeable H^{+} ($cmol_c\ kg^{-1}$)	-	-	1.02
Surface area ($m^2\ g^{-1}$)	-	74.6	-
Textural class	-	-	Silty loam (33% sand, 50% silt and 17% clay)



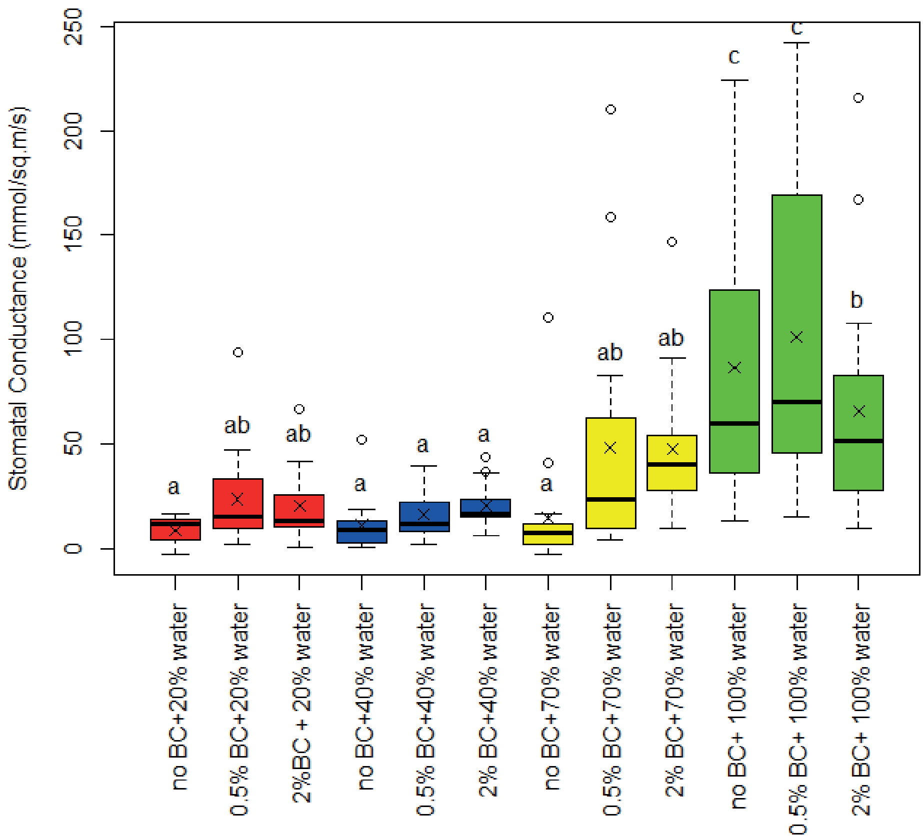
Supplementary Fig. 1

Effect of control, 0.5% biochar and 2% biochar irrigated with four different watering regimes (20%, 40%, 70% and 100% of 1000 ml water per pot per 5days) on average soil moisture content after second leaf emergence (15 d) to harvesting (50 d); mean ± sd, n=4.



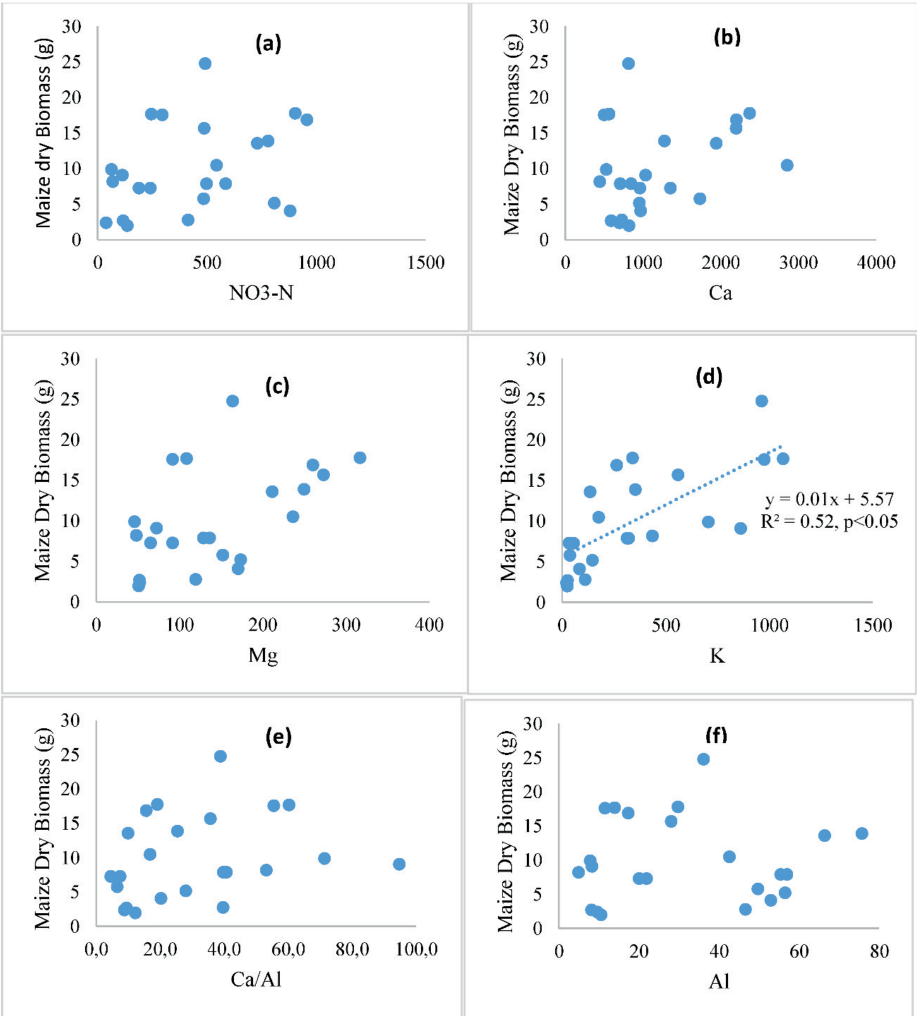
Supplementary Fig. 2

Relationship between average stomatal conductance vs soil moisture content and (*fig a*) average stomatal conductance vs maize dry biomass (*fig b*) under water stress experiments, n=12



Supplementary Fig. 3

Effect of combination of various biochar doses and watering rates on the stomatal conductance of maize leaves (mmol m^{-2} s^{-1}) at 50 d. Box and Whisker plot showing 1st quartile, 2nd quartile /median, and 3rd quartile data of stomatal conductance; 12 levels, n=16 (N=192). Sign (x) on the middle of the each box plot refer to the average maize stomatal conductance of each level/treatment. Different letters above the box plot indicate significant differences between treatments under water stress experiment.



Supplementary Fig. 4

Relationship between various soil nutrient supply rates and maize dry biomass under PRS treatments

Supplementary Table 2

Plant available phosphorous (soil extractable P) under water stress, nutrient stress and acid stress experiment. Different letters inside the table of each available phosphorous (P-AL) column represents significant differences between various treatments under water stress, nutrient stress and acid stress experiment following two way ANOVA (post hoc-tukey test, $P < 0.05$).

Treatments (water stress)	P-AL (mg/kg)[1]	Treatments (Nutrient stress)	P-AL (mg/kg)[2]	Treatments (Acid stress)	P-AL (mg/kg)[3]
no BC + 40ml water pot^{-1}	14.0 ± 1.6x	no BC + no NPK pot^{-1}	6.13 ± 0.92X	no BC + no lime pot^{-1}	12.5 ± 0.60a
0.5% BC + 40ml water pot^{-1}	25.9 ± 1.5y	0.5% BC + + no NPK pot^{-1}	16.6 ± 0.64Y	0.5% BC + no lime pot^{-1}	23.3 ± 2.6b
2%BC + 40ml water pot^{-1}	68.8 ± 1.3z	2%BC + + no NPK pot^{-1}	69.1 ± 4.74Z	2%BC + no lime pot^{-1}	65.3 ± 0.80c
no BC + 80ml water pot^{-1}	14.9 ± 0.5x	no BC + 0.39 g NPK pot^{-1}	6.6 ± 0.55X	no BC + 0.25 g lime pot^{-1}	13.3 ± 0.52a
0.5% BC + 80ml water pot^{-1}	23.9 ± 1.9y	0.5% BC + 0.39 g NPK pot^{-1}	19.03 ± 0.55Y	0.5% BC + 0.25 g lime pot^{-1}	17.5 ± 1.63b
2% BC + 80ml water pot^{-1}	72.3 ± 3.9z	2% BC 0.39 g NPK pot^{-1}	75.0 ± 0.50Z	2% BC + 0.25 g lime pot^{-1}	44.3 ± 0.83d
no BC + 140ml water pot^{-1}	12.8 ± 0.5x	no BC + 0.78 g NPK pot^{-1}	8.8 ± 1.20X	no BC + 0.75 g lime pot^{-1}	8.9 ± 0.92a
0.5% BC + 140ml water pot^{-1}	19.4 ± 1.1y	0.5% BC + 0.78 g NPK pot^{-1}	17.7 ± 1.07Y	0.5% BC + 0.75 g lime pot^{-1}	17.5 ± 0.83b
2% BC + 140ml water pot^{-1}	57.6 ± 2.2z	2% BC + 0.78 g NPK pot^{-1}	74.4 ± 5.7Z	2% BC + 0.75 g lime pot^{-1}	46.5 ± 1.14d
no BC + 200ml water pot^{-1}	11.1 ± 0.3x	no BC + 1.19 g NPK pot^{-1}	11.1 ± 0.3X	no BC + 4.5 g lime pot^{-1}	11.1 ± 0.3a
0.5% BC + 200ml water pot^{-1}	23.3 ± 0.2y	0.5% BC + 1.19 g NPK pot^{-1}	23.3 ± 0.2Y	0.5% BC + 4.5 g lime pot^{-1}	23.3 ± 0.2b
2% BC + 200ml water pot^{-1}	84.1 ± 2.0w	2% BC + 1.19 g NPK pot^{-1}	84.1 ± 2.0W	2% BC + 4.5 g lime pot^{-1}	84.1 ± 2.0e

[1]Available phosphorous under water stress experiment,

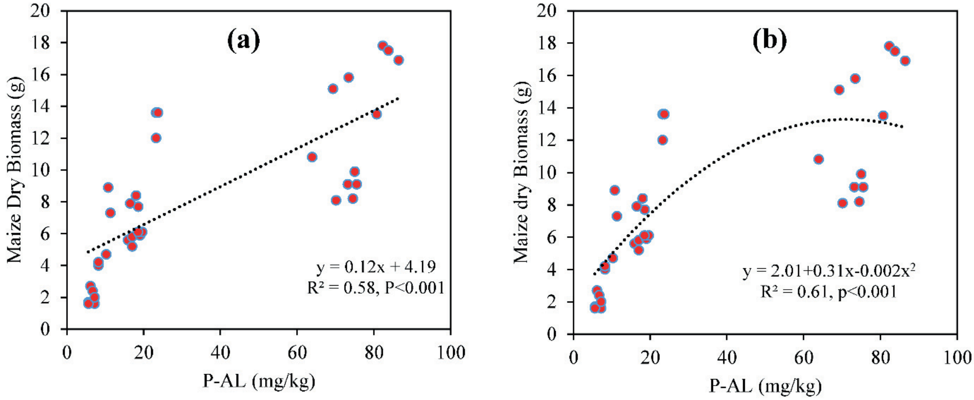
[2]Available phosphorous under nutrient stress and

[3]Available phosphorous under acid stress experiment.

Supplementary Table 3

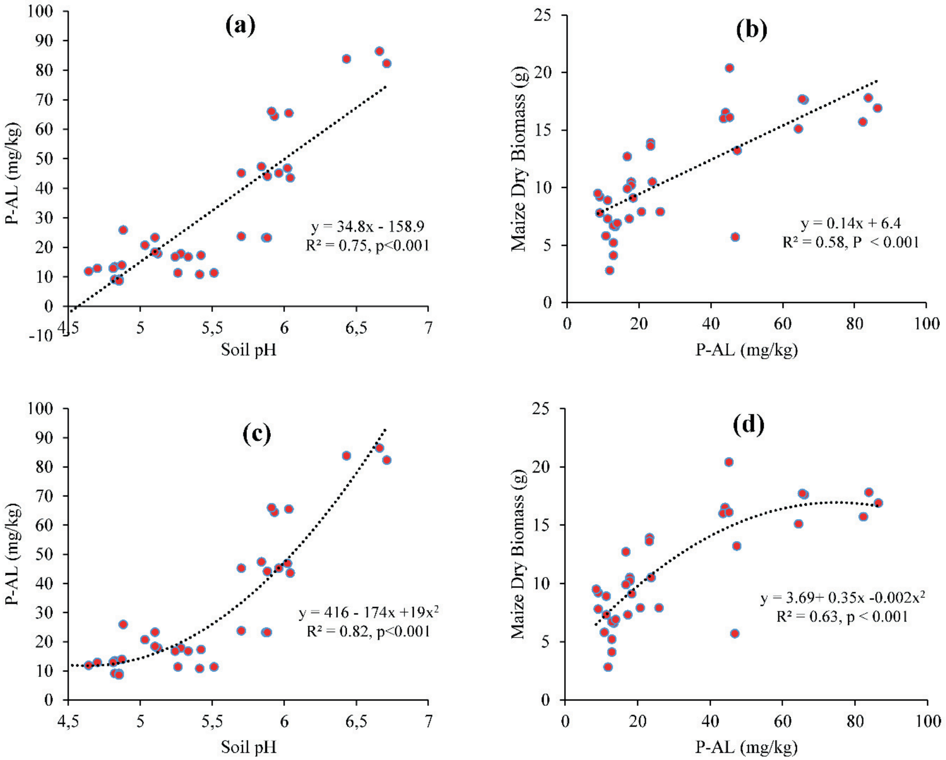
Effect of various biochar dosages and liming rates on in-situ soil pH on initial day (1d), half-way (24d) and final harvesting time (50 d). Average soil pH was calculated based on the observation on 1d, 24d and 50d, n=11. Different letters inside the table of each treatment on average soil pH (n=11) represents significant differences between various treatments following two way ANOVA (post hoc-tukey test, $P < 0.05$).

Treatments	Soil pH			Average soil pH
	1d (n=4)	24d (n=4)	50d (n=3)	n=11
no BC + no lime pot^{-1}	4.66 ± 0.06	4.62 ± 0.17	4.55 ±0.77	4.62 ± 0.09a
0.5% BC + no lime pot^{-1}	5.17 ± 0.2	4.99 ± 0.14	4.93 ± 0.06	5.05 ± 0.13bc
2%BC + no lime pot^{-1}	5.87 ± 0.04	5.96 ± 0.11	6.03 ± 0.05	5.94 ± 0.06d
no BC + 0.25 g lime pot^{-1}	4.86 ± 0.08	4.84 ± 0.05	4.77 ± 0.05	4.83 ± 0.03ab
0.5% BC + 0.25 g lime pot^{-1}	5.27 ± 0.13	5.23 ± 0.20	5.25 ± 0.06	5.27 ± 0.13c
2% BC + 0.25 g lime pot^{-1}	5.84 ± 0.19	5.90 ± 0.27	5.94 ± 0.05	5.90 ± 0.15d
no BC + 0.75 g lime pot^{-1}	4.90 ± 0.07	4.87 ± 0.04	4.78 ± 0.03	4.87 ± 0.06ab
0.5% BC + 0.75 g lime pot^{-1}	5.33 ± 0.26	5.28 ± 0.19	5.23 ± 0.05	5.29 ± 0.14c
2% BC + 0.75 g lime pot^{-1}	5.93 ± 0.11	5.98 ± 0.16	6.01 ± 0.09	5.98 ± 0.11d
no BC + 4.5 g lime pot^{-1}	5.42 ± 0.09	5.23 ± 0.32	5.41 ± 0.04	5.34 ± 0.15c
0.5% BC + 4.5 g lime pot^{-1}	5.88 ± 0.13	5.84 ± 0.19	5.83 ± 0.05	5.87 ± 0.13d
2% BC + 4.5 g lime pot^{-1}	6.41 ± 0.07	6.65 ± 0.29	6.72 ± 0.06	6.58 ± 0.13e



Supplementary Fig. 5

Linear regression (*fig a*) and quadratic regression (*fig b*) between plant available Phosphorous (P-AL) and maize dry biomass production for the nutrient stress experiment.



Supplementary Fig. 6

Linear regression between soil pH vs P-AL (*fig a*) and P-AL vs maize dry biomass production (*fig b*). Quadratic regression between soil pH vs P-AL (*fig c*) and P-AL vs maize dry biomass production (*fig d*) for the acid stress experiment.

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Paper IV.

Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth

Naba Raj Pandit, Hans Peter Schmidt, Jan Mulder, Sarah Elizabeth Hale, Olivier Husson, Gerard Cornelissen

Under review in Archives of Agronomy and Soil Science

Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth

Naba Raj Pandit [a,b,c*], *Hans Peter Schmidt* [d], *Jan Mulder* [b], *Sarah E. Hale* [a], , *Olivier Husson* [e,f,g], *Gerard Cornelissen* [a,b]

[a] Norwegian Geotechnical Institute (NGI), Oslo, Norway

[b] Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway

[c] Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal

[d] Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, 1974 Arbaz, Switzerland

[e] CIRAD, UPR AIDA, Avenue, Agropolis, F34398 Montpellier, France

[f] AIDA, Univ. Montpellier, CIRAD, Montpellier, France

[g] Africa Rice Center, BP 2031, 01 Cotonou, Benin

*corresponding author (navraj20@gmail.com)

Abstract: Biochar (BC) amendment to soil is widely recognized as a means to improve agricultural productivity and to mitigate climate change. Obtaining expensive, import based mineral fertilizer is a challenge for many tropical smallholder farmers. This work showed for the first time that organic nutrient transformation techniques based on locally available materials (manure, greenwaste, advanced biochar) can increase the fertilizing efficiency of the resulting substrate by a factor of three. Here, we use three different composting methods produced from raw materials (green waste and farmyard manure) to investigate the techniques of organic nutrient transformations; i) conventional composting (maturation without turning the piles), ii) aerobic composting (frequent turning) and iii) bokashi composting (anaerobic lacto-fermentation). Composting was carried out in the absence (compost alone) and presence of biochar (co-composted). Further, the substrates were compared to conventional mineral fertilization. Using biochar as an additive during composting may improve the fertilizer efficiencies of the resulting substrate. Biochar was produced locally in Nepal from an invasive forest shrub "*Eupatorium adenophorum*". A pot trial with maize grown in silty loam soil was carried out to investigate the agronomic effect produced using three above-mentioned methods, in the presence and absence of biochar. Biochar-compost mixtures were obtained using two processes; 1) co-composted (biochar mixed in to the compost during composting) and 2) post mixed (biochar and compost mixed together upon amendment i.e. mixed after composting). Significant effects especially of co-composted bokashi-biochar (60 t ha^{-1}) were observed on maize growth, which increased biomass by 243% compared to mineral NPK. Also co-composted bokashi-biochar showed better growth effects than amendments based on conventional and aerobic composting methods. Improved soil available nutrients (available P and other exchangeable base cations (K^+, Ca^+ and Mg^+)) were probably the cause of the superior growth effect of co-composted bokashi-biochar (lacto bacilli fermentation). The paper demonstrates that subsistence farmers in tropical countries can improve their on-farm organic nutrient management to achieve fertilizer efficiencies comparable or even better than mineral fertilizer.

Key words. Biochar, compost, co-composted biochar, bokashi fermentation, maize, Nepal

Highlights

- Organic nutrient transformation techniques in composts were investigated in the absence and presence of biochar additive (co-composted).
- Compost and co-composted biochar were produced from three composting methods; conventional, aerobic and lactic fermentation (bokashi).
- Co-composted bokashi-biochar significantly increased soil available P and exchangeable base cations (K^+, Ca^+, Mg^+).

- With co-composted bokashi-biochar subsistence farmers can improve their on-farm organic nutrient management to achieve fertilizer efficiencies better than mineral fertilizer

1. Introduction

Biochar amendment, either alone or in combined with organic or mineral fertilizers, to low productive tropical soils, has been recognized as an efficient and sustainable method to improve farm productivity (Lehmann and Rondon, 2006; Liang et al., 2006; Martinsen et al., 2014; Yamato et al., 2006). Biochar addition has resulted in improved soil physicochemical properties such as pH, cation exchange capacity (CEC), base saturation (BS) and water-holding capacity (Chan et al., 2008; Cornelissen et al., 2013; Glaser et al., 2002; Obia et al., 2016) as well as biological properties such as enhanced microbial activities (Atkinson et al., 2010; Ye et al., 2016).

Recently, biochar-compost mixtures have been investigated as a method to produce effective biochar-based slow release organic fertilizers (Schmidt, 2012; Ye et al., 2016). Biochar can either be mixed with composting materials during the composting process, i.e. "co-composted", or added directly to stored matured compost (Vandecasteele et al., 2016). Addition of biochar during the composting process changes the compost properties and quality and can lead to improved physicochemical properties (organic carbon content (OC), pH, moisture content) and nutrient availability (nitrogen, phosphorous and other important nutrients) in the end product (Agegnehu et al., 2016; Prost et al., 2013; Vandecasteele et al., 2016; Zhang and Sun, 2014). The co-composting process results in an organic coating on the biochar particles which reduces the hydrophobicity of biochar and improves nutrient retention conditions leading to improved agronomic performance (Hagemann et al., 2017; Joseph et al., 2017; Kammann et al., 2015). This organic coating on the biochar particles also may affect soil redox (Eh) status (Hagemann et al., 2017). Plants are affected by very low and very high Eh or pH and these parameters should be kept at a medium level for optimal performance (Husson, 2013). The application of highly oxidized co-composted biochar (with high Eh) could have positive agronomic impact on highly reduced soils, however, for oxidized conditions in aerobic soils, high Eh could negatively affect the soil-plant-microorganism system and crop production (Husson, 2013). In such cases, strongly reduced low-

Eh bokashi fermented biochar (lacto-fermentation) could have positive effect on highly oxidized soils thereby maintaining healthy soil ecosystem and better crop yield (Husson, 2013).

In a recent field study, the amendment of co-composted biochar to tropical Ferralsol increased maize crop grain and biomass production by 10-29% and 9-18% respectively when compared to inorganic fertilizers application (Agegnehu et al., 2016). In pot experiments, co-composted biochar (2% w/w) was observed to quadruple plant growth of *Chenopodium quinoa* in a nutrient poor sandy soil compared to the non-amended control (Kammann et al., 2015). Another recent field study (Schmidt et al., 2017) conducted in moderately acidic Nepalese silty loam soils demonstrated a significant agronomic benefit of biochar combined with organic fertilizers (cow urine and manure) when compared with biochar or organic fertilizer alone. In this trials, the average yield of various crops that received organic biochar based-fertilizers doubled compared to crops that received traditional organic fertilization and NPK-biochar fertilization respectively (Schmidt et al., 2017). However, the study of Schmidt et al. (2017) was primarily phenomenological and systematic and mechanistic trials to understand the agronomic effects of various biochar-compost formulations are currently lacking.

In addition, a further area currently left unexplored is the use of biochar in bokashi fermentation (anaerobic lactic fermentation), which uses manure and bio-waste products to produce high value soil amendments (Dreschke et al., 2015; Probst et al., 2015). The practice of Bokashi has been demonstrated in many farms worldwide, especially in Asia, for more than 30 years. Bokashi fermentation (Japanese term for "fermented organic matter") uses facultative anaerobic lacto bacilli bacteria to convert sugar into lactic acid, which results in improved growth, yield, quality and protection of vegetables and crops (Dou et al., 2012). These bacteria interact with the soil-plant environment in a complex manner to suppress plant pathogens and diseases and optimize soil nutrient availability. Most published studies (70%) reported a positive effect of such lactic fermented bokashi amendment on the growth of vegetables (Olle and Williams, 2013). Mixing biochar in to the manure and bio-waste products further improves the fermentation process, and the end product has been shown to have positive effects on plant available nutrients (available P) and crop yield (Andreev et al., 2016; Liu et al., 2012). However, no systematic research exists on the agronomic effect of lactic fermented bokashi-biochar mixtures.

In the present study, three different processes of composting, both in the presence and absence of biochar were tested; 1) Conventional composting; 2) Aerobic composting and 3) Bokashi fermentation. Composts were added to soils alone (Comp), together with biochar but added separately (Comp+$BC_{post-mix}$), and together with biochar after co-composting such that biochar and compost were added together (Comp+$BC_{co-comp}$). The three methods differ from each other, as conventional composting (Comp.conv) does not involve turning the piles, while aerobic composting (Comp.aer) involves turning the piles and bokashi fermentation (Bok) is an anaerobic lacto-fermentation process in a closed environment. This study is, to the best of our knowledge, the first in which organic nutrient transformations techniques in the presence of biochar additives were investigated and their effects on soil fertility and plant growth were assessed. Using biochar as an additive during composting process (co-composted) may increase the fertilizing efficiency of the nutrients. These co-composted organic amendments were compared with inorganic amendments (both in presence and absence of biochar) and with compost alone. This paper aimed at demonstrating that subsistence farmers in tropical countries may improve their on-farm organic nutrient management to achieve fertilizer efficiencies comparable or even better than expensive, imported based mineral fertilizers. The experiment was performed under greenhouse conditions with maize plantation in a silty loam Inceptisol from Rasuwa, Nepal. Controlled conditions were chosen since the main purpose of the present study was to obtain a mechanistic understanding of biochar-compost effects produced from three composting process on soil fertility.

Biochar used in this experiment was produced from the invasive, non-palatable feedstock, *Eupatorium,* ubiquitous in Africa (Mshandete and Parawira, 2009) as well as South and East Asia (Liu et al., 2006). Biochar produced form this feedstock has previously demonstrated beneficial agronomic effects in both pot (Pandit et al., 2017) and field (Schmidt et al., 2015) trials for a moderately acidic silty loam soil from Nepal. In the present study, we hypothesized that the biochar-compost mixtures, especially co-composted biochar, when compared with inorganic treatments and compost alone could 1) enhance soil available nutrients (mainly P and K); and 2) increase maize biomass growth as a result of the increased soil nutrient availability in this soil.

2. Materials and methods

2.1. Composting methods

The raw materials used for the composting process were green waste (mixed vegetable and *Eupatorium* waste in the ratio of 20:80, chopped to 3-5cm length), cattle farmyard manure (FYM) and biochar (BC). Green waste was collected from agricultural farmland and manure from a cattle farm located at Pathik Foundation, Kathmandu Valley, Nepal. Biochar was produced from "*Eupatorium adenophorum*" feedstock using a flame curtain steel shielded soil pit "Kon- Tiki" kiln with a pyrolysis temperature of 600-700°C (Cornelissen et al., 2016). The elemental content of *Eupatorium* was 42.9% C, 1.4% H and 1.5% N (Pandit et al., 2017). The biochar had a pH_{CaCl2} of 8.9, CEC of 121 $cmol_c\ kg^{-1}$ (measured with 1M NH_4NO_3 extraction) and an organic carbon content of 71.4 % (Pandit et al., 2017).

Three composting methods were used to produce organic based fertilizer formulations; 1) conventional composting (Comp.conv), 2) aerobic composting (Comp.aer) and 3) bokashi fermentation (Bok). For all three methods, raw materials (green waste: manure ratio of approx.1:1.5 w/w wet weight) were mixed thoroughly in the absence and presence of biochar (10% vol.). Addition of 10% biochar during composting or matured stored compost has been shown to be optimal for making biochar-based organic fertilizers (Kammann et al., 2016). During this process, 144 kg of chopped green waste and 224 kg manure were mixed to make homogenous mixtures and separated into two equal portions (184 kg each), after which 16 kg wet biochar was added to one portion (equivalent to 10% by volume, 6 kg dry biochar). The portion without biochar was separated into three heaps for conventional composting (46 kg, 16.5 kg dry weight), aerobic composting (92 kg, 33kg dry weight) and bokashi fermentation (46 kg, 16.5 kg dry weight). The portion with biochar (co-composted) was also divided into three heaps with the same mass as for composting without biochar; conventional composting with biochar (Comp.conv+$BC_{co-comp}$), aerobic composting with biochar (Comp.aer+$BC_{co-comp}$) and bokashi biochar fermentation (Bok+$BC_{co-comp}$). Conventional co-compost, aerobic co-compost and bokashi co-compost heaps received 1.5 kg, 3 kg and 1.5 kg dry biochar respectively, which was equivalent to 10% vol. biochar (Table S1 and Image S1).

Both heaps for conventional composting (with and without biochar) were stored at the same location and the entire composting process was completed without turning the piles (Misra et al., 2003). Under aerobic composting, 5 kg of clay soil (wet weight) collected from a rice paddy field were added to both of the heaps (with and without biochar) and mixed thoroughly to ensure better aeration. Both aerobic composting heaps were kept in the shade of a shelter to provide protection from rainfall and to ensure optimum humidity conditions required for good quality compost. Aerobic compost was matured by manual turning the composting piles daily for the first three weeks, and every three days after that (Hagemann et al., 2018). For bokashi fermentation, raw materials from two heaps (with and without biochar) were placed on two separate plastic sheets in layers (6 layers in total for each heap). Thus, each layer of bokashi and bokashi-biochar fermentation received 2.75 kg and 3 kg of raw materials (dry weight equivalent). Before adding each of the next layers, 150 g sugar (900 g of sugar in total) was applied along with 100 ml spray of diluted fermentative liquid (1:20 parts; 600 ml in total) followed by the compaction of each layer with the help of a ram. The fermentative liquid was prepared in 1.5 L bottles where 300 ml fermentative liquid from the previous batch and 30 g fresh mixed leaves were added to 1 L of water. This starter blend was anaerobically fermented for 10 days. Both plastic sheets were entirely closed and soil was placed on the top of the sheets to ensure anaerobic conditions. Bokashi fermentation involves lacto-bacilli activity under anaerobic condition to break down the organic substrates (Andreev et al., 2016). All three composting processes lasted for 80 d (11th July - 29th September, 2016).

2.2. Physicochemical characterization of compost

2.2.1. Monitoring during composting.

During the composting process, moisture content (% vol.), temperature (^{0}C) and redox potential (mV) were measured every 7 d until compost maturation. pH of compost and co-composted BC-compost from conventional and aerobic composting piles were measured at day 40 and day 80. For practical reasons the anaerobically packed bokashi fermentation systems, moisture content, temperature, Eh and pH were monitored only once, after harvesting of the product (80 d). Moisture content (% vol.) was measured (three measurement per pile) by a hand-held Time-Domain Reflectometer (TDR; SM150 soil moisture sensor, Delta T devices Ltd, Burwell, Cambridge, England). Composting piles were watered when they had moisture contents less than 40% (measured with TDR), to prevent a decrease of microbial activity. Compost pH was measured in 0.01M $CaCl_2$ solution (1:5 solid-water ratio) with a WTW pH 320 device. Eh (mV) was measured with WTW equipment with an AgCl reference electrode (combined 3M AgCl electrode) and corrected to standard hydrogen electrode (SHE) as a function of temperature. The temperature of composting piles were recorded with temperature sensor rods.

2.2.2. Compost characterization

Compost and co-composted BC-compost samples were collected (after compost harvest) randomly from different portions within each heap for chemical analysis. The methods used to determine available (ammonium lactate extractable) phosphorus (P-AL), and total P and K analysis are described in Pandit et al. (2018), and outlined in the supplementary information (Description S1).

2.3. Greenhouse experiment design

A pot trial was carried out under greenhouse conditions for 55 d (from 12^{th} October - 8^{th} December, 2016) at Matatirtha, Kathmandu, Nepal (N 27° 41' 51", E 85° 14' 0", altitude 1520 m). Average temperature recorded inside the greenhouse during the trial period was 23.5 ^{0} C (average minimum 15 ^{0} C and average maximum 32 ^{0} C, n=50). Nursery plant pots (top, middle and bottom diameter; 24 cm, 19 cm and 12 cm respectively and 20 cm high) with approx. 6 L volume were filled with 3 kg of air-dried silty loam (Inceptisol) that was collected from arable soil, Rasuwa district, Nepal (27^{0}, 59,479' N and 85^{0}, 11,987' E), as described in Pandit et al. (2018). No crowding of roots inside the pots was observed, in line with field experiments where root-to-shoot ratios of maize plants in similar soils were in the order of 2-5%, and root systems weighed in the order of 10-20 g (Abiven et al., 2015).

The experiment consisted of 88 pots (N=88) in a completely randomized design and included 22 treatments with four replications each (n=4). An overview of the treatments included in the greenhouse trial is presented in Table S2.

Three types of compost i.e. conventional compost, aerobic compost and bokashi fermentation with premixed or co-composted biochar (Comp.conv+$BC_{co\text{-}comp}$, Comp.aer+$BC_{co\text{-}comp}$ and Bok+$BC_{co\text{-}comp}$), post mixed biochar (Comp.conv+$BC_{post\text{-}mix}$, Comp.aer+$BC_{post\text{-}mix}$ and Bok+$BC_{post\text{-}mix}$) and without biochar (Comp.conv, Comp.aer and Bok) were applied in two different dosages (40 g per pot and 120 g per pot equivalent to 20 t ha^{-1} and 60 t ha^{-1} respectively) resulting in 18 treatments. In addition to these, four additional treatments were tested; (1) NPK equivalent to nutrient content in 20 t ha^{-1} compost (0.12 g N, 0.06 g P_2O_5 and 0.24 g K_2O), (2) NPK equivalent to nutrient content in 60 t ha^{-1} compost (0.36 g N, 0.18 g P_2O_5 and 0.72 g K_2O), (3) NPK equivalent to nutrient content in 20 t ha^{-1} compost + 3 g biochar and (4) NPK equivalent to nutrient content in 60 t ha^{-1} compost + 9 g biochar). By assuming a 15% N availability, 30% P and K availability in the compost (Kammann et al., 2016), the amount of NPK content in 20 t ha^{-1} and 60 t ha^{-1} compost was calculated (calculated and shown in Table S2). Mineral nutrient NPK was applied in the form of Urea for N, orthophosphate for P_2O_5 and murate of potash for K_2O.

After mixing all the organic and inorganic amendments thoroughly in the respective treatment, three maize seeds (*Manakamana-4 variety*) were sown 2 cm below the soil surface in each pot. Upon germination and emergence of two leaves (14 d), the smaller and least robust plants, selected based on visual observation, were removed from the pots to leave one plant for the experimental duration. All the pots were irrigated daily with 140 ml water pot^{-1} day^{-1} until second leaf emergence (14 d) after which the pots were irrigated every five days at 700 ml water pot^{-1} until harvest. These watering rates are representative of the growth season in Nepal (Pandit et al., 2018). Pots were rotated every four days until harvest to ensure homogeneity of the treatments (exposure to sunlight, shade, humidity etc.). Weeding was carried out twice (30 d and 42 d) during the experiment.

2.4. In-situ soil physicochemical analysis

Soil moisture content (% by vol.) was measured every five days until harvest (55 d) following exactly the same procedure as described in Pandit et al. (2018). Soil redox potential (Eh) was measured at 30 d and 55 d with the same device used for in-situ compost (Eh) measurement. Measured E (mV) was corrected to SHE as a function of temperature.

2.5. Plant harvest and soil analysis

Maize plants were harvested on day 55 and fresh weight of above ground biomass (AGB) was measured immediately after harvest. Maize AGB was oven dried at 70^0C for 24 hours to calculate the dry weight (g).

Soil sample were collected at 1 d, 30 d and 55 d of pot trial and analyzed for soil pH (0.01M $CaCl_2$ solution at a 1:5 solid to water ratio). For all other soil tests, soil samples were collected after maize plants were harvested. Soil from all individual pots was collected to make a bulk composite sample for each of the 22 treatments. Dried (105^0 C; 12 hours) and sieved (2 mm) soil samples were analyzed for CEC, exchangeable acidity (H^+) and plant available phosphorous (P-AL) following Pandit et al. (2018), and as outlined in the supplementary information (Description S2).

2.6. Statistical analysis

Data were analyzed using R statistical software version 3.2.2. Normality and homogenous variances of all data sets were tested with Sharpio-Wilk -and Levene's test, respectively. One factor ANOVA was used to assess the effect of three different processes of composting (with and without BC) on composting quality (aeration, moisture content, temperature) and the available nutrients in the matured compost. Likewise, one factor ANOVA was used to assess the effect of various organic (compost) and inorganic amendments (NPK) with and without biochar (categorical explanatory variable) applied at two different dosages (20 t ha^{-1} and 60 t ha^{-1}) on soil physicochemical properties and maize biomass production (response dependent variable). REG-WQ (Ryan / Einot and Gabriel / Welsch test procedure) post hoc test ($P=0.05$) was used to evaluate the significant differences between various treatment means. The differences between treatments were significant at $P < 0.05$, unless otherwise stated. A linear regression model was used to assess the correlation between maize biomass production and the various soil parameters (pH, Eh, nitrate, ammonium, P-AL, K^+, Ca^+ and Mg^+).

3. Results

3.1. Composting conditions

The average moisture content was 5-15% higher for biochar-amended composts than for non-biochar composts for all three composting systems throughout the composting period (Fig.1a). Recorded temperatures were in the range of the mesophilic phase (below 40^0C) but a thermophilic phase (above 40^0C) was not reached, neither for compost nor for biochar co-compost in the conventional and aerobic composting piles (Fig.1b). Similar to moisture content, average Eh was around 50mV higher for biochar-amended composts than for non-biochar composting ones (Fig 1c). The pH of composting piles measured at day 40 and day 80 did not show significant variation, therefore, the values were averaged to give one reading for each of the compost and co-composted piles. Aerobic co-composted biochar (Comp.aer+BCco-comp) had the highest pH (7.9 ± 0.1) and bokashi fermentation (Bok) showed the lowest pH (pH 4.89 ± 0.04) (Fig S1). By contrast, bokashi in the presence of biochar (Bok-BC) was neutral (pH 7.20± 0.02). Previous work (Probst et al., 2015) has demonstrated that lactic acid fermentation occurred at neutral pH.

Fig. 1a,b,c. Average moisture content, temperature and Eh of different composting piles (y-axis) measured at every 7 day until compost harvest (x-axis), n=3.

3.2. Nutrient content of composts and co-composted biochar-composts

Total K and P and available P were higher for bokashi fermentation (Bok and Bok+$BC_{co\text{-}comp}$) compared to the other two composting processes (Table 1). Inorganic N contents (NO_3^- and NH_4^+) were observed to be higher for conventional (Comp.conv) and aerobic composts (Comp.aer) than bokashi fermentation

(Bok). Bok+BC$_{co\text{-}comp}$ fermentation substrate contained higher NO_3^- (61.0 ± 1.5 mg kg^{-1}) compared with bokashi fermentation in absence of biochar (32.01 ± 0.08 mg kg^{-1}) (Table 1).

Table 1. Nutrient content of different composts and co-composted biochar-composts (mean ± sd). Different letters within the column of each compost nutrient content (response variable) represents significant differences between various composting types (with and without biochar amendments) following one way-ANOVA (post hoc REG.QW test, $p < 0.05$).

3.3. Biomass production

Bokashi applied at 60 t ha^{-1} in the presence (but not the absence) of biochar showed a strong positive effect on maize biomass production, especially after co-composting (Fig.2). Bok+BC$_{co\text{-}comp}$ significantly increased biomass production per pot (5.93 ± 0.71 g) by 243%, 204% and 149% compared with NPK (1.73 ± 0.57 g), NPK+BC (1.95 ± 1.42 g) and bokashi without biochar (2.38 ± 0.46 g) respectively (Fig.2). Bok+BC$_{post\text{-}mix}$ also showed increased biomass production (4.03 ± 0.93 g) compared with NPK and NPK+BC, but the effect was less pronounced so (+132 % and +106%, respectively; Fig.2). Compost and BC-compost produced from conventional and aerobic systems showed no significantly different biomass production from NPK (control) and NPK+BC (Fig.2). None of the composts and or co-composted compost-biochar formulations showed any significant differences from NPK and NPK+BC treatments at the application rate of 20 t ha^{-1} (Fig S2).

Fig. 2. Effect of various organic and inorganic amendments in the presence and absence of biochar applied at the rate of 60 t ha^{-1} composts on maize biomass production (mean ± SE, n=4). Different letters above a bar of each treatment represents significant differences between various treatments following one way-ANOVA (post hoc-REG-WQ test, $P < 0.05$).

3.4. Soil properties after the trial

Available P in post-trial soil was significantly higher for biochar-compost mixtures (both co-compost and post-mix BC-compost applied at 60 t ha^{-1}) produced using all three composting methods (44 to 105 mg kg^{-1}) when compared with NPK and NPK+BC treatments (34 and 38 mg kg^{-1} respectively) (Table 2). Much higher P-AL was observed for Bok+$BC_{co-comp}$ (105 mg kg^{-1}) than for all other organic amendments with and without biochar. No differences between Bok+$BC_{co-comp}$ and other organic and inorganic amendments were observed on soil P-AL when applied at the rate of 20 t ha^{-1} (Table S3). Soil NO_3^- was significantly increased upon amendment with Bok+$BC_{co-comp}$ and Bok+$BC_{post-mix}$ compared with bokashi without biochar applied at 60 t ha^{-1} (Table 2).

Soil CEC was significantly increased for all biochar-composts mixtures applied at 60 t ha^{-1} (8.4 to 12 $cmol_c$ kg^{-1}) compared to NPK with and without biochar (7.8 $cmol_c$ kg^{-1}) (Table 2). All compost and BC-composts showed higher pH and lower amounts of exchangeable Al^{3+} compared with NPK treatment (Table 2). However, even the Al^{3+} in the NPK treatment was below levels where effects on plant roots can be expected (around 0.2 $cmol_c$ kg^{-1}) (De Wit et al., 2001).

The average soil moisture content (% vol.) measured by daily TDR (n=32) was significantly increased for Bok+$BC_{co-comp}$ (17 ± 2 %) and Bok+$BC_{post-mix}$ (16 ± 2 %) compared with other organic and inorganic amendment when applied at 60 t ha^{-1} (Table 2) but not at 20 t ha^{-1} (Table S3).

Table 2. Effect of organic amendments (compost and co-compost) mixed with and without biochar and applied at 60 t ha^{-1} on soil physicochemical properties. Different letters within each column denotes significant differences between treatments on soil properties following one-way ANOVA (REG-WQ test, $p < 0.05$).

4. Discussion

4.1. Composting conditions.

The addition of biochar during aerobic composting under the shelter resulted in optimal moisture content (> 40 % vol, required for effective microbial activity) for longer periods compared with the non-biochar aerobic piles (Fig 1a). This was mainly due to increased water holding capacity resulting from the amendment of biochar, and supports previous studies (Kammann et al., 2016; Pandit et al., 2018). For conventional and bokashi fermentation piles, higher moisture levels were also observed for biochar amended composts (Fig 1a). Similarly, Eh was higher for biochar-amended compost throughout the composting period compared to non-biochar composting piles, possibly due to the higher porosity of biochar that maintain the higher oxygen level for longer periods (Kammann et al., 2016). However, the measured values of Eh were slightly lower (below 500mV) (Fig 1c) than is normally expected following biochar addition (Eh > 500 mV), but were still in the range required for good soil quality (Husson, 2013). Among all compost and co-composted types, bokashi fermented compost and co-compost had significantly higher amount of available nutrients (P-AL, K and NO_3^-) (Table 1). In accordance with this, Boechat et al. (2013) reported accelerated organic matter degradation upon bokashi fermentation that enhanced available mineral nutrients in the system, and thus could reduce the requirement of nutrient supplements (John et al., 2007). Bokashi fermentation in the presence of biochar ($Bok+BC_{co-comp}$) had higher amounts of NO^{3-} than bokashi without biochar (Bok) (Table 1), which could possibly be explained by the higher Eh in $Bok+BC_{co-comp}$.

4.2. Soil physicochemical properties and available nutrients

In addition to improved soil CEC and base cations (K^+, Ca^{2+}, Mg^{2+}), soil P-AL was found to be highest for the co-composted biochar from bokashi fermentation (60 t ha^{-1}) (Table 2). Indeed, the ratio of available P to total P (P-AL/total P) where total P was equal for all bokashi fermentation with and without biochar (Table 1), follows the order: Bok < $Bok+BC_{post-mix}$ < $Bok+BC_{co-comp}$ (Fig 3). Co-composted biochar from aerobic compost ($Comp.aer+BC_{co-comp}$) also had higher soil P-AL and K^+ contents compared with aerobic compost without biochar (Comp.aer) and NPK treatments, which supports the earlier observations of increased soil nutrients availability (available P and K) following co-composting with biochar (Kammann et al., 2015; Prost et al., 2013). However, the availability of nutrients in soil with aerobic and conventional compost in the presence of biochar were far less than those observed for bokashi fermented biochar amendments. This may be due to the lacto bacilli amended during bokashi fermentation that enhanced microbial organic degradation which in turn increased nutrient availability in the soil system (Boechat et al., 2013). In addition, during bokashi fermentation, most of the nutrients are preserved in the hermetic fermentation pack, unlike conventional (open condition) and aerobic composting (piles sheltered with roof top but open from side) that were subjected to nutrient leaching and elemental losses in the rainy season (aerobic piles mainly affected by lateral rainfall) during which composting took place (Hagemann et al., 2018). Beneficial effects of co-composted biochar on soil physicochemical properties and available nutrients (P-AL, K^+, NO_3^-) have previously been reported by Agegnehu et al. (2016). Increased nutrient retention could be due to the formation of organic coating in co-composted biochar, which entrap or adsorb dissolved nutrients in the system (Hagemann et al., 2017; Joseph et al., 2017; Kammann et al., 2015).

Fig .3. Ratio of soil available P and total P (P-AL/tot P) for bokashi fermentation in the absence and presence of biochar; mean ± sd, n=3.

4.3. Maize biomass (AGB) production

In this study various organic amendments (with and without biochar) did not demonstrate significant effects on maize biomass production with the exception of bokashi-biochar, where positive effects were especially prominent after co-composting (Fig 2). In accordance with this, Andreev et al. (2016) reported significantly higher maize height following the amendment of bokashi fermented-biochar mixtures compared to a control, mineral fertilizers and other organic amendments (stored faeces, stored cattle urine and stored urine) in field trials in loamy eroded soils. This reflects the positive effect of co-composted biochar-bokashi (Bok+$BC_{co\text{-}comp}$), which has significant growth promoting features compared with biochar and compost alone (Kammann et al., 2016). This is possibly due to the activity of lacto bacilli in bokashi fermentation that increases the amount of available nutrients which results in improved crop growth, quality and yield (Dou et al., 2012). In accordance with this, Agegnehu et al. (2016) reported beneficial effects following the amendment of co-composted biochar on soil available nutrients, and a subsequent positive effect on crop growth and development

Among various soil factors explored as a function of organic and inorganic amendments (60 t ha^{-1}) on maize biomass production, soil P-AL ($R^2 = 0.55$) and exchangeable base cations such as K^+ ($R^2 = 0.64$), Ca^{2+} ($R^2 = 0.35$) and Mg^{2+} ($R^2 = 0.36$) stood out and showed significant positive relationships ($P<0.001$) with maize biomass production (Fig.4). However, statistically significant positive relationship between these soil parameters (P-AL, K^+, Ca^{2+} and Mg^{2+}) and maize biomass were only observed when bokashi/biochar mixtures were included (Fig.S3). In addition, other soil factors such as soil NO_3^-, NH_4^+, pH and Eh did not show significant positive correlation with biomass production (Fig.S4). Measured mineral N (NO_3^-, NH_4^+) at the end of the experiment could provide an indicator for available N and its relationship with maize biomass production. However, in our previous study with similar soil and crop under greenhouse conditions (Pandit et al., 2018) available mineral N measured via in-situ plant root simulators (nutrient supply rates with cation and anion probes buried in soil) was not correlated with maize biomass production, illustrating no effect of soil available N on maize biomass in this soil. The relationship between soil moisture content and maize biomass was not investigated, as the measured moisture content (Table 2) was relatively low for all the treatments including bokashi-biochar mixtures (ranging from 8 to 17 % vol.), and this variable was not considered as a potential soil factor for improved crop growth. Thus, the relatively high maize AGB production (at least double that of all other additions; Fig.2) of the co-composted bokashi-biochar formulation can possibly be explained by higher soil available nutrients such as P-AL, K^+, Ca^{2+} and Mg^{2+} in this soil (Table 2, Fig.4). Optimal maize growth requires P-AL to be in the range of 50-80 mg kg^{-1} (Krogstad et al., 2008). Most of the organic amendment (including co-composted biochar from aerobic and conventional compost) and inorganic amendments used in this work had soil P-AL < 55 mg kg^{-1}, with the exception of Bok-$BC_{co\text{-}comp}$ (> 70 mg kg^{-1}), providing a possible explanation for the superior effects on crop growth that were observed for bokashi fermentation in presence of biochar (Table 2). Indeed, P deficiency symptoms were observed for many of the treatments including bokashi without biochar but not for bokashi-biochar formulations. In our previous pot trial with the same soil and crop type, P-AL was one of the most important growth limiting factors, and it was effectively alleviated upon biochar amendment (increased P-AL from 11 mg kg^{-1} at control to 84 mg kg^{-1} at 2% w:w biochar addition) (Pandit et al., 2018). Furthermore, Bok+BC$_{co\text{-}comp}$ also improved soil CEC mainly through increased exchangeable base cations such as K^+, Ca^{2+} and Mg^{2+} (Table 2), which all contributed to the beneficial effect observed for biomass production (Fig.4). There are many previous studies that have observed that the amendment of biochar results in higher amounts of exchangeable base cations especially K^+. These studies have concluded that the effects resulted in positive effects on crop production (Agegnehu et al., 2016; Martinsen et al., 2014; Yamato et al., 2006).

In addition, the reduced Eh of Bok-BC$_{co\text{-}comp}$ (-71.31 ± 59.00 mV) amended to oxidized soil (> 400 mV) could lead to improvements of the soil-plant-microorganism system (Husson, 2013) and thus a concurrent increases in biomass production. A factor contributing to the lack of positive agronomic effects of conventional and aerobic compost/biochar formulations may be that a thermophilic phase was not reached, with temperatures in the range of 60-70 °C. The reason for this was possibly that the compost piles used here were of a relatively small size.

Fig. 4. Relationship between P-AL and exchangeable base cations (K^+, Ca^{2+} and Mg^{2+}) with maize biomass for various organic and inorganic treatments applied at 60 t ha^{-1} of composts.

In addition to improved soil nutrient availability, bokashi fermentation that involve lacto bacilli activity may further increase soil (micro) biological and enzymatic activity in the presence of biochar, which could then have beneficial effects on soil biogeochemical cycles and plant pest/diseases and therefore result in an improvement of crop growth (Lehmann et al., 2011). Increased soil biological activity is most likely to be due to the sorption capacity of biochar that inactivates growth inhibitors (such as monoterpenes) or soil contaminants in the soil system (Hale et al., 2015; Lehmann et al., 2011). In conclusion, *hypothesis 1 that biochar-compost formulations could enhance soil available nutrients (mainly P and K)* was accepted for aerobic and bokashi co-composting but rejected with regard to conventional co-composting. *Hypothesis 2 that maize biomass growth would be increased as a result of this increased soil nutrient availability* was only accepted for bokashi-biochar mixtures especially co-composted biochar-bokashi formulations. Hypothesis 2 was rejected for conventional and aerobic biochar-compost formulations.

Set in a wider perspective, this work showed for the first time that organic nutrient transformation techniques based on locally available materials (manure, greenwaste) can increase the fertilizing efficiency of the resulting substrate by a factor of three, especially when including biochar. A possible limitation of bokashi-biochar co-composting formulations could be that they were only effective at high compost addition rates of 60 t ha^{-1}, but not at usual compost dosages of 20 t ha^{-1}. The high 60 t ha^{-1} dosage was used in order to gain a better understanding of the processes operating. More work is needed to find out whether the positive effect of adding bokashi-biochar formulations encompasses many soil types, or whether the effect was specific for the presently studied oxidized Inceptisol, where a high dosage was needed to improve the crop growth. The results shown here for maize may not be fully representative for other plants, and may vary with soil type and required available nutrient for proper growth and development. The improved crop growth for bokashi fermentation in the presence of biochar was probably partly explained by increased nutrient availability (most notably P), possibly mediated by lacto bacilli which can further increase plant nutrient availability and organic matter turnover. Other effects of the lactic acid bacteria, such as on pathogens and other soil biota, were not studied here, and should be focused on in subsequent work. The present study investigated effects in related to a limited range of soil physical and chemical parameters, but detailed microbiological and spectroscopic studies are needed to mechanistically unravel the effects of bokashi-biochar formulations.

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Supplementary Information

Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth

Naba Raj Pandit [a,b,c], Hans Peter Schmidt [d], Jan Mulder [b] , Sarah E. Hale [a], , Olivier Husson [e,f,g], Gerard Cornelissen [a,b]*

[a] Norwegian Geotechnical Institute (NGI), Oslo, Norway

[b] Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway

[c] Nepal Agroforestry Foundation (NAF), Koteshwor, Kathmandu, Nepal

[d] Ithaka Institute for Carbon Strategies, Ancienne Eglise 9, 1974 Arbaz, Switzerland

[e] CIRAD, UPR AIDA, Avenue, Agropolis, F34398 Montpellier, France

[f] AIDA, Univ. Montpellier, CIRAD, Montpellier, France

[g] Africa Rice Center, BP 2031, 01 Cotonou, Benin

*corresponding author (navraj20@gmail.com)

Description S1: characterization of compost samples

Samples were oven dried at 40 ^{0}C for three days and passed through a 2 mm sieve prior to analysis. For total P and K analysis, compost samples (0.25g) were first decomposed in ultrapure nitric acid using an ultraclave at 260 ^{0}C and at a pressure of about 50 bars. After decomposition, samples were diluted to 50 ml with deionized water and analyzed through microwave assisted nitrogen plasma instrument (Agilent 4200) via selective atomic lines (213.618 nm for P and 769.897 nm for K). For NO_3^- and NH_4^+ analysis, samples were extracted with 2M KCl solution (5 g dry compost added to 25 ml of 2M KCl solution; 1:5 solid to solution ratio). This solution was shaken (100 rpm) for 30 min, filtered through pre-washed blue ribbon filters (Whatman 589/3), and was introduced in a flow injection system (FIA star 5000) for analysis. Available (ammonium lactate extractable) phosphorus (P-AL) was measured according to (Krogstad et al., 2008). In this process, 2 g dry compost was added to 40 ml ammonium lactate solution, filtered (Whatman filter paper) and diluted ten times. Ascorbic acid (0.4 ml) and molybdenum reagent (0.4 ml) were added to the diluted samples and standards. Measurements were done using spectrophotometry (Pandit et al., 2017).

Description S2: Soil analysis

For soil CEC measurement, samples were extracted with 1M NH4NO3 at pH 7and the exchangeable cation concentrations were determined using ICP-OES. Exchangeable acidity (H+) was determined by titration the extract with 0.02 M NaOH to pH 7. Plant available phosphorus (P-AL) was measured similar to the compost analysis using the ammonium lactate method (Krogstad et al., 2008). For soil NO3- and NH4+ measurement, fresh samples were extracted within 12 h with 2M KCl solution (20 g dry soil added to 50 ml of 2M KCl solution and measured with a flow injection system (FIA star 5000), similar to the compost analysis.

Image S1. Biochar added at the rate of 10% vol. in the composting mixtures (conventional composting pile).

Table S1. Quantity of raw materials used for making different compost.

	Composting methods	Raw materials					
		Fresh weight (kg)			Dry weight (kg)		
		GW	FYM	BC	GWM	FYM	BC
i	Comp.conv	18	28	-	7.5 (42)	9 (32)	-
ii	Comp.conv + BCco-comp	18	28	4	7.5 (42)	9 (32)	1.5
iii	Comp.aer	36	56	-	15 (42)	18 (32)	
iv	Comp.aer + BCco-comp	36	56	8	15 (42)	18 (32)	3
v	Bok	18	28	-	7.5 (42)	9 (32)	-
vi	Bok + BCco-comp	18	28	4	7.5 (42)	9 (32)	1.5

Note: Figure inside the parenthesis of the column (dry weight GWM and FYM) are the dry matter % of GWM and FYM.

Table S2. Overview of the treatments in the greenhouse experiment

Treatment no.	Description of the treatments	Comp.dry wt (g per pot)	BC dry wt (g per pot)	N (g per pot)	P_2O_5 (g per pot)	K_2O (g per pot)	Urea (g per pot)	H_3PO_4 (g per pot)	MOP (g per pot)
1	NPK ~ 20t/ha compost[1]	-	-	0.12	0.06	0.24	0.26	0.08	0.4
2	NPK ~ 60t/ha compost[2]	-	-	0.36	0.18	0.72	0.78	0.24	1.2
3	NPK ~ 20t/ha compost + 3t/ha BC	-	6	0.12	0.06	0.24	0.26	0.08	0.4
4	NPK ~ 60t/ha compost + 9t/ha BC	-	18	0.36	0.18	0.72	0.78	0.24	1.2
5	Comp.conv (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
6	Comp.conv (60t/ha)	120	-	0.36	0.18	0.72	-	-	-
7	Comp.conv+BCpost-mix (20t/ha)	40	4	0.12	0.06	0.24	-	-	-
8	Comp.conv+BCpost-mix (60t/ha)	120	12	0.36	0.18	0.72	-	-	-
9	Comp.conv+BCco-comp (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
10	Comp.conv+BCco-comp (60t/ha)	120	-	0.36	0.18	0.72	-	-	-
11	Comp.aer (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
12	Comp.aer (60t/ha)	120	-	0.36	0.18	0.72	-	-	-
13	Comp.aer+BCpost-mix (20t/ha)	40	4	0.12	0.06	0.24	-	-	-
14	Comp.aer+BCpost-mix (60t/ha)	120	12	0.36	0.18	0.72	-	-	-
15	Comp.aer+BCco-comp (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
16	Comp.aer+BCco-comp (60t/ha)	120	-	0.36	0.18	0.72	-	-	-
17	Bokashi (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
18	Bokashi (60t/ha)	120	-	0.36	0.18	0.72	-	-	-
19	Bokashi+BCpost-mix (20t/ha)	40	4	0.12	0.06	0.24	-	-	-
20	Bokashi+BCpost-mix (60t/ha)	120	12	0.36	0.18	0.72	-	-	-
21	Bokashi+BCco-comp (20t/ha)	40	-	0.12	0.06	0.24	-	-	-
22	Bokashi+BCco-comp (60t/ha)	120	-	0.36	0.18	0.72	-	-	-

[1] **20t/ha compsot:** 20 000 kg/ha compost with approx. 2% N, 2% K_2O, 0.5% P_2O_5 is 400:100:400 N:P_2O_5:K_2O. Assume 15% N availability, 30% P_2O_5 and K_2O availability. Thus available NPK (agronoimcal not elemental) rates approximately 60:30:120 kg/ha. Pots (3 kg) contain 500 000 times less soil than 1 ha (10 cm depth, BD 1.5: 1 500 000 kg). Thus, per pot *0.12 g N, 0.06 g P_2O_5, 0.24 g K_2O*. This is 0.26 g urea, 0.08 g H_3PO_4, 0.4 g MOP per pot.

[2] **60t/ha compost:** 60 000 kg/ha compost with approx. 2% N, 2% K_2O, 0.5% P_2O_5 is 1200:300:1200 N:P_2O_5:K_2O. Assume 15% N availability, 30% P_2O_5 and K_2O availability. Thus available NPK rates (agronomical not elemental) approximately 180:90:360 kg/ha. Pots (3 kg) contain 500 000 times less soil than 1 ha (10 cm depth, BD 1.5: 1 500 000 kg). Thus, per pot *0.36 g N, 0.18 g P_2O_5, 0.72 g K_2O*. This is 0.78 g urea, 0.24 g H_3PO4, 1.2 g MOP per pot.

Table S3. Effect of organic (compost) amendments mixed with and without biochar applied at the rate of 20 t ha^{-1} on soil chemical properties. Different letters within each column denotes significant differences between treatments on soil properties following one-way ANOVA (REG-WQ test, P < 0.05).

Treatments	In-situ soil properties			Ex-situ soil characterization, n=3									
	Moisture	Eh	pH	Mg^{2+}	Ca^{2+}	K^+	Al^{3+}	H^+	CEC[1]	BS	P-AL	NO_3^--N	NH_4^+
	(%), n=32	(mV), n=8	n=12	Cmolc$_c$ Kg^{-1}						(%)	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
NPK	8 ± 1a	522 ± 14ab	5.6 ± 0.3a	1.4 ± 0.04a	4.8 ± 0.2ab	0.8 ± 0.01c	0.08 ± 0.01d	0.55 ± 0.54abcd	7.7 ± 0.3b	90 ± 7abc	28.3 ± 1.8de	4.6 ± 0.6ab	7.7 ± 1.1a
NPK + BC[2]	11 ± 1b	546 ± 9bc	5.9 ± 0.3ab	1.3 ± 0.08a	4.6 ± 0.0a	0.5 ± 0.01a	0.10 ± 0.01d	0.00 ± 0.00a	6.7 ± 0.1a	98 ± 0d	24.0 ± 1.4c	6.0 ± 0.6b	6.2 ± 1.2a
Comp.conv	11 ± 1b	521 ± 17ab	6.1 ± 0.1b	1.4 ± 0.08a	4.5 ± 0.2ab	0.5 ± 0.05a	0.08 ± 0.01d	0.09 ± 0.11a	6.6 ± 0.2a	97 ± 2cd	16.5 ± 1.1a	4.9 ± 1.9a	7.9 ± 4.2a
Comp.conv+BC$_{post-mix}$	11 ± 1b	533 ± 13ab	6.3 ± 0.1bc	1.5 ± 0.08a	4.9 ± 0.2b	0.5 ± 0.01a	0.06 ± 0.00c	1.44 ± 0.13e	8.5 ± 0.4bc	82 ± 1a	19.9 ± 1.4b	6.5 ± 4.6ab	7.6 ± 3.8a
Comp.conv+BC$_{co-comp}$	11 ± 1b	525 ± 12ab	6.3 ± 0.2bc	1.7 ± 0.04bc	5.5 ± 0.0c	0.5 ± 0.02a	0.05 ± 0.0b	0.97 ± 0.21d	8.7 ± 0.2cd	88 ± 2b	28.8 ± 5.1cde	3.7 ± 0.6a	9.6 ± 2.8a
Comp.aer	11 ± 1b	515 ± 8a	6.3 ± 0.1bc	1.9 ± 0.01d	5.9 ± 0.1d	0.5 ± 0.02a	0.03 ± 0.02ab	0.28 ± 0.05b	8.6 ± 0.1c	96 ± 1c	27.3 ± 1.3d	5.7 ± 3.8ab	8.3 ± 2.9a
Comp.aer+BC$_{post-mix}$	12 ± 2b	514 ± 10a	6.5 ± 0.1c	1.9 ± 0.12d	5.6 ± 0.2cd	0.6 ± 0.04a	0.01 ± 0.02a	0.14 ± 0.13a	8.3 ± 0.4bc	98 ± 2cd	30.0 ± 1.4e	4.5 ± 1.3ab	8.6 ± 5.4a
Comp.aer+BC$_{co-comp}$	13 ± 2b	555 ± 7c	6.5 ± 0.1c	1.6 ± 0.04b	5.0 ± 0.0b	0.5 ± 0.02a	0.06 ± 0.01bc	1.65 ± 0.05f	9.0 ± 0.1d	80 ± 1a	25.9 ± 1.4cd	3.4 ± 0.9a	8.1 ± 2.7a
Bok	10 ± 1ab	512 ± 8a	6.1 ± 0.1b	1.5 ± 0.11ab	4.3 ± 0.3a	0.7 ± 0.03b	0.04 ± 0.03abc	1.40 ± 0.17de	8.0 ± 0.3bc	81 ± 2a	25.0 ± 1.8cd	4.7 ± 0.8ab	7.8 ± 4.1a
Bok$_{-+}$BC$_{post-mix}$	11 ± 2ab	543 ± 9b	6.3 ± 0.2bc	1.6 ± 0.01b	4.8 ± 0.2ab	0.7 ± 0.03b	0.05 ± 0.01bc	0.51 ± 0.05c	7.7 ± 0.3b	92 ± 1b	19.3 ± 0.3b	4.6 ± 1.7ab	7.7 ± 3.1a
Bok$_{-+}$BC$_{co-comp}$	11 ± 2ab	548 ± 10bc	6.3 ± 0.1bc	1.7 ± 0.01c	5.1 ± 0.2bc	0.8 ± 0.07bc	0.03 ± 0.01ab	0.05 ± 0.06a	7.8 ± 0.4b	98 ± 1cd	30.6 ± 5.5cde	5.3 ± 3.0ab	8.5 ± 2.3a

[1] CEC measured as the sum of exchangeable cations and extractable acidity (H^+); Na^+ was also included for the CEC calculation (not shown in the table)

[2] Biochar applied at the rate of 3 t ha^{-1}

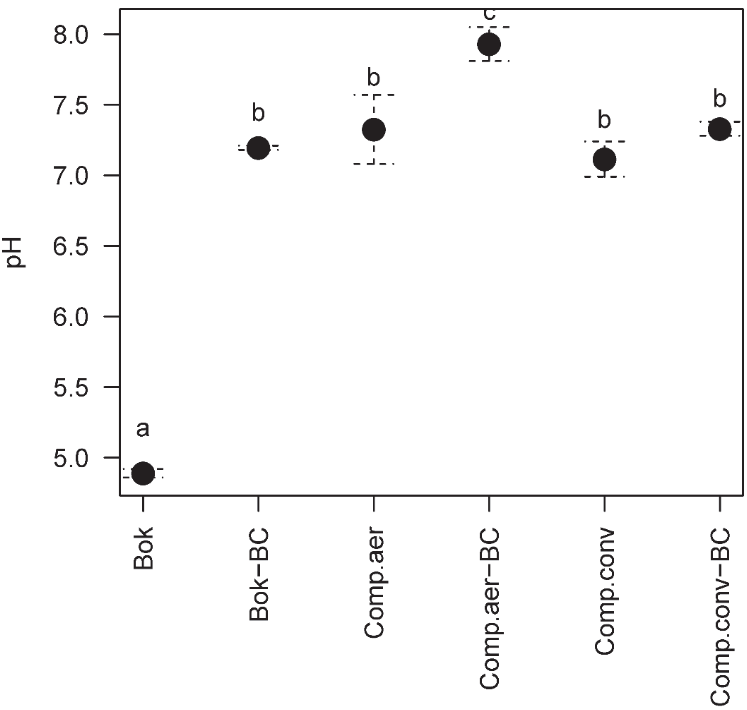

Fig.S1. Average pH of composting piles, n=2 measured at day 40 and day 80.

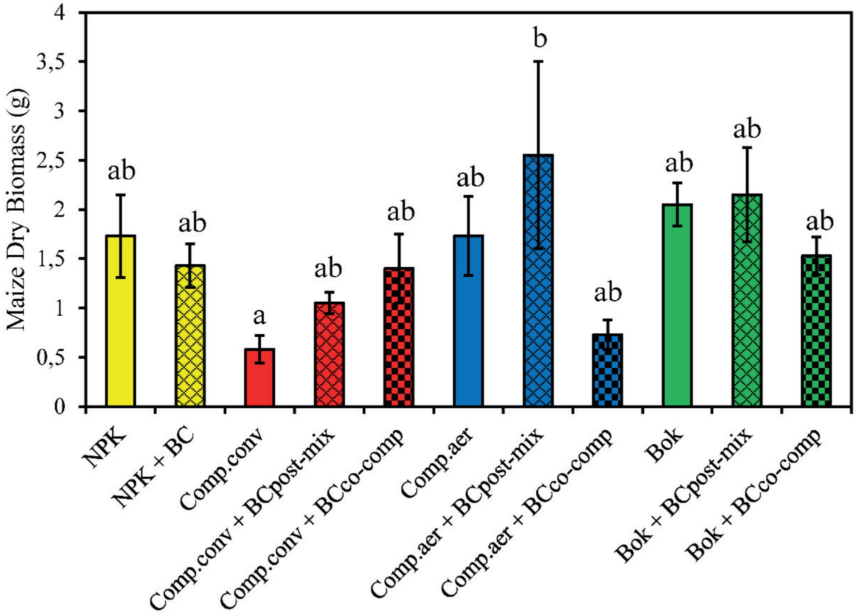

Fig.S2. Effect of various organic and inorganic amendments in the presence and absence of biochar applied at the rate of 20 t ha^{-1} composts on maize biomass production (mean ± SE, n=4). Different letters above a bar of each treatment represents significant differences between various treatments following one way-ANOVA (post hoc-REG-WQ test, $P < 0.05$).

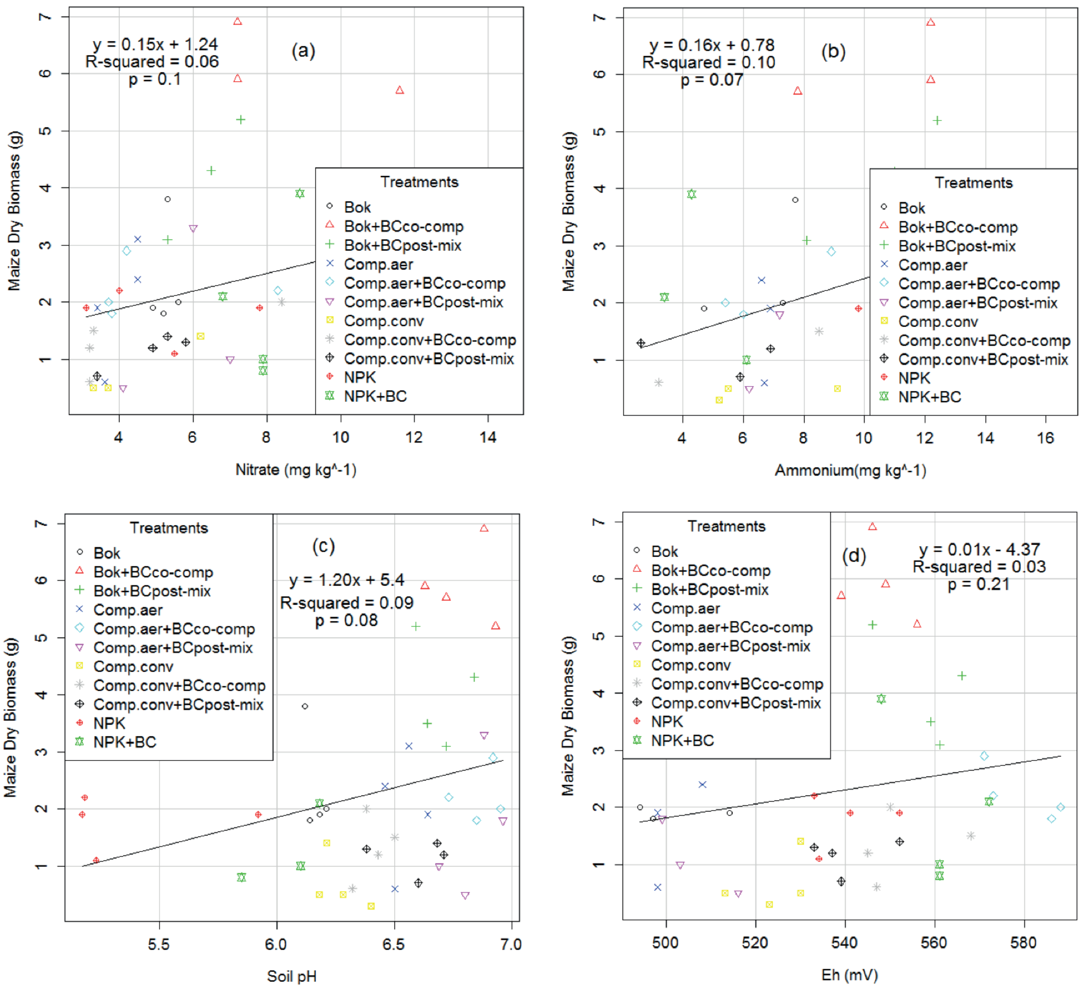

Fig.S3. Relationship between soil parameters (NO_3^-, NH_4^+, pH, Eh) and maize biomass for various organic and inorganic treatments applied at 60 t ha^{-1} composts

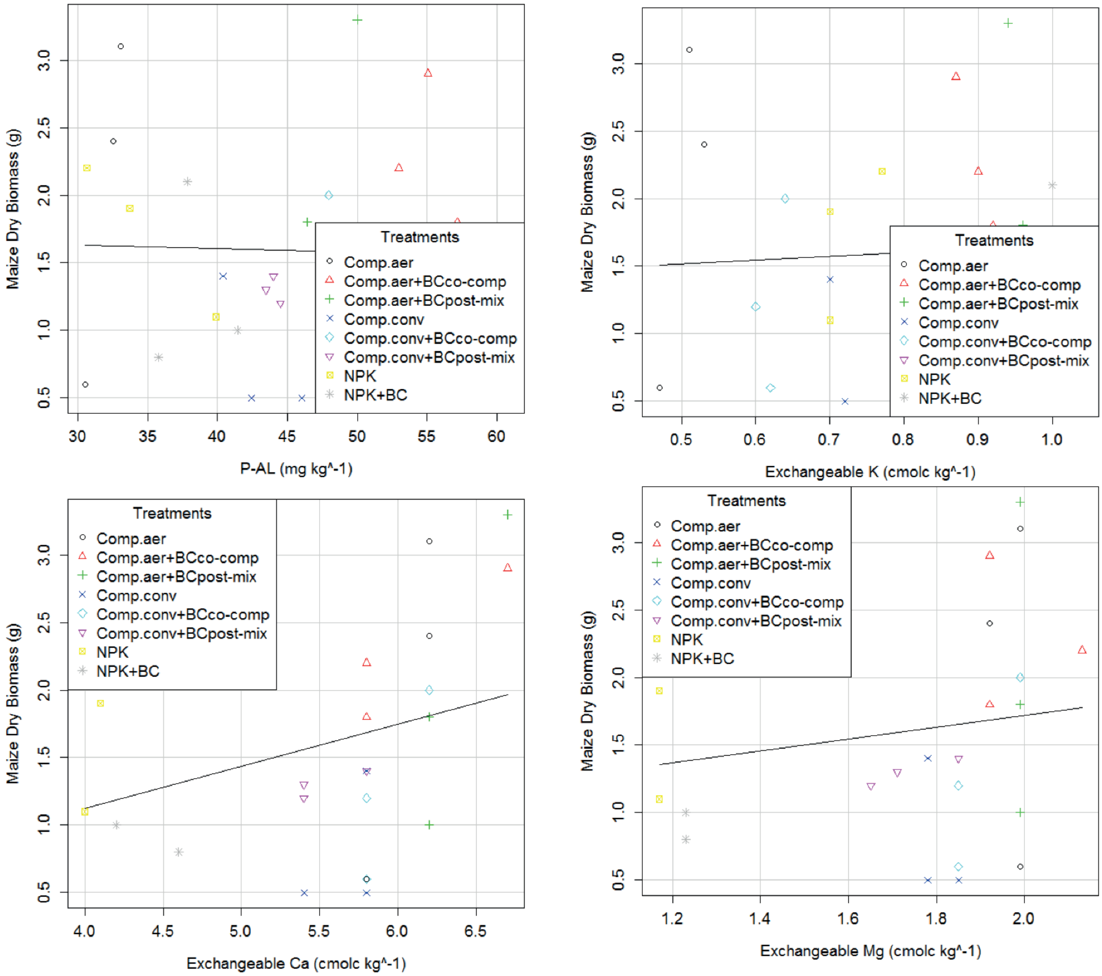

Fig.S4. Relationship between P-AL and exchangeable base cations (K^+, Ca^{2+} and Mg^{2+}) with maize above dry biomass for various organic and inorganic treatments applied at 60 t ha^{-1} of composts excluding bokashi compost, co-compost and post-mixed biochar. None of the explored soil parameters showed significant linear relationship ($p>0.05$) with maize biomass.

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Paper V.

Multi-year double cropping biochar field trials in Nepal: finding the optimal dosage through agronomic trials and cost-benefit analysis

Naba Raj Pandit, Jan Mulder, Sarah Elizabeth Hale, Andrew R Zimmerman, Bishnu Hari Pandit, Gerard Cornelissen

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Multi-year double cropping biochar field trials in Nepal: finding the optimal dosage through agronomic trials and cost-benefit analysis

Naba Raj Pandit[a,b,c], Jan Mulder[b] , Sarah E.Hale[a], Andrew R. Zimmerman[d] , Bishnu Hari Pandit[e], Gerard Cornelissen[a,b]

[a] *Norwegian Geotechnical Institute (NGI), Oslo, Norway*

[b] *Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway*

[c] *Nepal Agroforestry Foundation (NAF), Kathmandu, Nepal*

[d] *University of Florida, Department of Geological sciences, Gainesville, FL, USA*

[e] *Kathmandu Forestry College (KAFCOL), Kathmandu, Nepal*

* Corresponding author. Email: navraj20@gmail.com

Abstract

Poor water and nutrient retention are the major soil fertility limitations in low productivity agricultural soils of Nepal. Addition of biochar is one of the ways to overcome these hindrances. In the present study, six different biochar doses (control, 5 t ha^{-1} , 10 t ha^{-1} , 15 t ha^{-1}, 25 t ha^{-1} and 40 t ha^{-1}) were amended to a moderately acidic silty loam soil from Rasuwa, Nepal and the effects on soil physicochemical properties and maize and mustard yield over three years (i.e., six cropping seasons) were investigated. Biochar addition did not show significant effects on maize and mustard grain yield in the first year but significant positive effects were observed during the second and third. During the second year, maize grain yield significantly increased by 50%, 47% and 93% and mustard grain yield by 96%, 128% and 134% when at 15 t ha^{-1}, 25 t ha^{-1} and 40 t ha^{-1} of biochar was amended respectively. A similar significant trend in yield of both crops was observed in the third year. Yields for both maize and mustard correlated significantly with plant available P, K^+, pH, total OC%, CEC, base saturation (*p<0.001*) , and increased as a function of biochar addition.

On the basis of the measured crop yields for the various biochar dosages, a cost-benefit analysis was done, and gross margin was calculated to optimize biochar dosage under local farming practices. Total cost included financial cost (farm input, labor and biochar production cost), health cost, and carbon emission cost during biochar production. Total income comprised sale of crops and carbon sequestration credits. The cost-benefit analysis indicated the optimal biochar dosage to be 15t ha^{-1} for all C price scenarios, increasing gross margin by 10% and 42%, respectively, under 0 and 42 US$ per ton CO_2 price scenarios.

Key words: Eupatorium, biochar, soil quality, crop yield, cost-benefit analysis, Nepal

Highlights

- Field trials with six different biochar doses were done in a silty loam soil, Nepal
- Maize-mustard field cropping system was applied over three years (six seasons)
- Biochar addition showed effects on crop growth during the second and third year.
- Crop yield was positively correlated with plant available P and K
- Cost-benefit analysis included health cost, climate cost/benefit and agronomic cost/benefit
- Optimal biochar dosage was 15 t ha^{-1} from agronomic and economic perspective

1. Introduction

Biochar, the carbonaceous product from pyrolysis of biomass (Lehmann, 2007) has received much interest as it is able to abate two major global challenges, i.e., sustainable enhancement of soil fertility and climate change mitigation (Chan et al., 2008; Lehmann et al., 2006). Several studies have confirmed significant improvement of soil chemical properties such as increased soil pH, cation exchange capacity

(CEC), exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and soil organic carbon (SOC) upon biochar addition to soil (Chan et al., 2008; Lehmann and Rondon, 2006; Liang et al., 2006; Martinsen et al., 2014; Yamato et al., 2006). In addition, biochar amendment has shown positive effects on plant available water (Herath et al., 2013; Mukherjee and Lal, 2013; Obia et al., 2016) and microbial activity (Atkinson et al., 2010). Biochar has a recalcitrant nature and remains stable in soil for many years, thus, acting as an effective C sequestration technique combating climate change (Lehmann et al., 2006; Zimmerman, 2010). However, biochar addition does not have a uniform effect on soil fertility and carbon stability, and may vary with feedstock, pyrolysis condition, soil, climate, and crop type where biochar was applied (Manyà, 2012).

A meta-analysis carried out by Jeffery et al. (2017), reported an average increase in crop yield of 25% mainly due to liming (increased pH) and nutrient addition effects of biochar in low fertile tropical soils. In a sentinel study from Sumatra, Indonesia, addition of 20 t ha^{-1} increased soil pH (from 3.9 to 5.1) and reduced Al^{3+} concentration from 2.67 $cmol_c$ kg^{-1} (toxic level for plant growth) to 0.12 $cmol_c$ kg^{-1} (Yamato et al., 2006). Increased pH upon biochar addition increases phosphorus bioavailability (P-AL) (Hale et al., 2013). Biochar addition also directly adds K^+ to tropical soils (Martinsen et al., 2014; Pandit et al., 2018), while soil nitrogen has been observed to be higher in biochar-amended soils due to reduced leaching and absorption of N into biochar pores (Kammann et al., 2015; Laird et al., 2010).

The majority of biochar-crop effect studies has been performed in field trials or pot trials for only one cropping cycle. However, longer-term studies with more cropping cycles are needed to examine the yield effect of biochar addition (Griffin et al., 2017).

Despite positive agronomic and environmental effect of biochar amendment in tropical soils (Jeffery et al., 2017), there are very few studies where explicit attempts were undertaken to analyze the feasibility of the biochar project i.e. agronomic, environmental and financial benefit of using biochar under small-scale normal farmer agriculture practice (Joseph, 2009). Financial return is often the main indicator used by farmers when they make decisions related to whether or not to adopt biochar amendment in their cropping system (Bach et al., 2016). Inadequate analysis of detailed cost-benefit effectiveness of biochar project may deter from using biochar as a soil amendment (Joseph, 2009; Pratt and Moran, 2010). In addition, it is not easy to convince farmers from developing regions to adopt new farming practices (Bach et al., 2016). For example, though clean burn flame curtain kiln charring, was introduced in Indonesia, farmers were reluctant to use this technology unless its financial and agronomic returns were made clear (Smebye et al., 2017). Low profit was derived when agronomic values only were taken into account (Bach et al., 2016). Higher economic returns are fetched when soil carbon sequestration benefits of biochar are considered, which offsets biochar production cost including negative environmental and health impact cost (gas and aerosols emissions) during biochar making (Sparrevik et al., 2014).

In Nepal, low soil productivity (low P, organic C, base saturations, CEC) and a shortage of fertilizer has severely affected the status of crop production (Brown et al., 1999; Schreier et al., 1994). Farmers have poor access to chemical fertilizers and imported mineral fertilizers are quite expensive (Shrestha and Pandit, 2017). To cope with such challenges, it is essential to develop more efficient soil management strategies that increase crop production per unit of land (Brown et al., 1999). One alternative to overcome such limitations could be the application of biochar and farm yard manure (FYM) (Schmidt et al., 2017).

So far, the effect of biochar on soil fertility and crop production has been studied only for a single cropping season in Nepalese soil (Schmidt et al., 2015, 2017). In addition, research on the optimal biochar dosage and socio-economic aspects is scarce. We aimed at filling this knowledge gap by studying the long-term fertility and economic effects of *Eupatorium* biochar amendments in a six-season trial for two crops (maize and mustard), at six different dosages in a moderately acidic silty loam soil of Rasuwa, Nepal. Biochar addition in this soil has shown positive effects on maize biomass production under greenhouse pot experiment, and the reason behind this was found to be mainly due to improved nutrient retention capacity (available P and K^+) (Pandit et al., 2017). In the present study, the optimal biochar dosage was examined both with regard to agronomic effectiveness and financial profitability. To address the profitability, a cost-benefit analysis was carried out on the basis of the observed crop yield, including health and climate costs of gas and aerosol emissions from biochar production, as well as C sequestration benefits, using a variety of carbon prices from zero to full social cost of carbon (EPA, 2013). Not many studies have taken into account both the climate cost of methane emissions and the health cost of aerosol emissions during biochar production. Biochar stability was also investigated via a limited number of benzopolycarboxylic acid (BPCA) analyses, which represent the condensed aromatic C (pyrogenic carbon) content of soil.

2. Materials and Methods

2.1. Study area

The experimental site was located in Dhaibung/Nilkantha village development committee of Rasuwa district, Nepal (28^0, 10'', 0' N and 85^0, 11'', 0' E) at an altitude of 1378 m above sea level (Fig.S1). Rasuwa is 115 km north of Kathmandu. The study area receives 1850 mm average annual rainfall (receiving highest precipitation in June/July and lowest in November/December) and mean annual temperature of 15.4 ^{o}C (Rasuwa district profile, 2013). The study area is situated in the central development region, a part of Bagmati zone, where common agronomic cereal crops encompass maize, mustard and wheat.

2.2. Biochar production

The invasive ubiquitous forest shrub *"Eupatorium adenophorum"* about 1-2 m high and with stems up to 2 cm thick was used as a feedstock for biochar production. Eupatorium is unpalatable to livestock and has shown negative impact on livelihood sustainability, food security and ecosystem management (Kunwar, 2003). In this study, *Eupatorium* feedstock was collected from community forest areas, farm uplands/lowlands and bank of the river (Image S1). Elemental analysis of the Eupatorium from *EuroEA Elemental Analyzer* showed 42.9% C, 1.4% H and 1.5% N (Table 1). Biochar was produced with a traditional earth mound kiln (Image S2) with pyrolysis temperature of 450 - 500^0C. Subsequent research has shown that cleaner and simpler methods exist to make biochar, such as the flame curtain kiln technology (Cornelissen et al., 2016; Pandit et al., 2017).

2.3. Experimental set up and cultivation practices

A private farm (Image S2) with low pH (4.6) and CEC (6.4 $cmol_c$ kg^{-1}) in rainfed uplands was selected (Table 1). Twenty-four plots of 10 m^2 each were established on a flat area without shading trees, with 1 m spacing between plots. Six treatments with four replications (n=4) were assigned in four blocks in completely randomized design (CRD). Six different biochar dosages were used; 0 kg, 5 kg, 10 kg, 15 kg, 25 kg and 40 kg per 10 m^2 plot, equivalent to 0 ton ha^{-1} (control), 5 ton ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1}, 25 t ha^{-1} and 40 t ha^{-1} respectively. Higher dosages (25 t ha^{-1} and 40 t ha^{-1} biochar) are little realistic and are included only for scientific reasons. All treatments including control received equal amounts of mineral fertilizer N (in the form of urea; 60 kg N ha^{-1} after 60 d) and farmyard manure (a composted mixture of cow manure and greenwaste, 30 t ha^{-1} wet weight) according to farmers practice. During land preparation, biochar and manure were spread evenly followed by tillage (15 cm soil depth) and harrowing practices in all treatment plots. Terracing and drains were built in the side of the plots to conserve the top soil of each plot and to prevent erosion. The field trial was set up in April 2014. Each year, maize was grown in the wet season (April to August) followed by mustard in the dry season (September to February). This cropping pattern (maize-mustard) was continued for three years (until February 2017). Biochar was applied only once at the onset of the trials (April 2014).

After a week of land preparation, maize seed (*Arun variety*) was sown at a depth of 5-6 cm following 30 cm x 30 cm spacing within each treatment plot. Hand weeding was carried out twice (30 d and 60 d). Upon maturity, maize plants were harvested manually and a month after maize harvest, mustard seeds were broadcast in equal quantity in all 24 plots. Manure and N were applied each year during maize cultivation (first, third and fifth season) but not in the following cropping season (second, fourth and sixth season with mustard) according to farmers practice.

2.4. Soil sampling and analysis

Before trial establishment, the soil was analyzed for pH, CEC, total organic carbon % (OC %) and nitrogen (N %) (Table 1). After the fifth season harvest, soil samples from all treatment plots were collected to make a composite soil sample. Soil samples were oven dried at 105^0 C for 24 hours, passed through a 2 mm sieve and crushed (< 2 mm) prior to analysis. Soil pH was measured in both water and 0.01M $CaCl_2$ (1:2.5, solid to solution ratio) using an Orion 1 Ross pH electrode. For CEC, soil was extracted with 1M NH_4NO_3 at pH 7 and the individual exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+ and Al^{3+}) were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Exchangeable H^+ was determined by titration the extract with 0.02 M NaOH to pH 7. Total CHN analysis was done with a CHN analyzer (LECO, Truspec).

In order to explore the stability of the biochar under field conditions, soil from the control and 40 t ha^{-1} field aged biochar plots, along with the fresh non-aged biochar, were subjected to BPCA analysis following the methods of Brodowski et al. (2005) and Dittmar (2008) with modification. Briefly, samples were digested in 4 M trifluoroacetic acid (TFA, 105°C, 4 h) to remove metals and polyvalent cations. Residue were then extracted in 0.5 mL of 65% HNO_3 at 170°C for 8 h under high pressure, then purified using Dowex cation exchange resin (50W, 200−400 mesh). Finally, BPCA compounds (B3CA to B6CA with 3 to 6 carboxyl group substituents, respectively) were identified via HPLC-DAD using certified standards.

Plant available phosphorus (P-AL, mg kg^{-1}) and plant available water (PAW, % vol.) were measured in three plots (control, 10 t ha^{-1} and 40 t ha^{-1}) to assess the effect of biochar addition on P availability and water retention capacity respectively. Other biochar doses were not included due to practical reason. Biochar dose at 10 t ha^{-1} was included to understand the mechanism of biochar on P-AL and PAW, which is applicable at local farmers cropping practice (≤ 15 t ha^{-1} biochar). Another higher dose (40 t ha^{-1} biochar) which is relatively less applicable at farmer's agricultural practice was included for scientific mechanistic understanding of biochar effect and comparison with other biochar dosages. Available (ammonium lactate extractable) phosphorus (P-AL) was measured according to Krogstad et al. (2008). For PAW measurement, hand packed soil samples were saturated and soil water measured at different matrix potentials (pF 2, field capacity and pF 4.2, wilting point) via ceramic pressure plates (Martinsen et al., 2014; Obia et al., 2016).

2.5. Biochar and FYM characterization

Biochar samples were analyzed in the same way as soil samples, and analyzed for pH, CEC and total CHN. Furthermore, biochar surface area was determined on a Quantachrome Autosorb1 surface area analyzer. N_2 adsorption isotherms were measured at 77 K and interpreted using Brunauer, Emmet, and Teller (BET) theory. The biochar used in this experiment had a pH $_{CaCl2}$ of 9.3, organic carbon content of 70%, CEC of 72 $cmol_c$ kg^{-1} and surface area (BET) of 74.6 m^2 g^{-1} (Table 1).

Manure samples were analyzed for total CHN % following similar procedure as operated for soil samples. For total elemental P and K analysis, manure samples (0.25g) were first decomposed in ultrapure nitric acid using an ultraclave at 260 ^{0}C and at a pressure of about 50 bars. After decomposition, samples were diluted to 50 ml with deionized water and analyzed through microwave assisted nitrogen plasma instrument (Agilent 4200) via selective atomic lines (213.618 nm for P and 769.897 nm for K). Manure had 30 % organic carbon, 1.6 % total N, 6.2 g kg^{-1} P and 25.3 g kg^{-1} K (Table 1).

Table 1

Characterization of Eupatorium feedstock, biochar, manure and soil used in the field trial

Properties	Feedstock	Biochar	Manure	Soil[1]
pH_{CaCl2}	-	9.3	-	4.6
pH_{H2O}	-	-	-	5.1
CEC ($cmol_c$ kg^{-1})	-	72	-	6.4
BS[2] (%)	-	-	-	74
Ca^{2+} ($cmol_c$ kg^{-1})	-	18	-	3.2
Mg^{2+} ($cmol_c$ kg^{-1})	-	13	-	1.2
K^+ ($cmol_c$ kg^{-1})	-	36	-	0.2
Al^{3+} ($cmol_c$ kg^{-1})	-	-	-	0.7
Ca/Al ratio	-	-	-	4.5

Total organic C %	42.9	70	30	1.6
Total H %	1.4	1.1	0.9	0.48
Total N %	1.5	0.46	1.6	0.18
Total P($g\ kg^{-1}$)	-	-	6.2	-
Total K ($g\ kg^{-1}$)	-	-	25.3	-
Available P $mg\ kg^{-1}$	-	-	-	12
Surface area	-	74.6	-	-
Textural class	-	-	-	Silty loam[3]
Order	-	-	-	Inceptisol

[1] Soil test before operating field trial experiment

[2] Base saturation

[3] Silty loam with 33% sand, 50% silt and 17% clay

2.6. Crop yield analysis

Upon maturity, maize and mustard plants were harvested manually from all the plots on the same day. Maize and Mustard above ground biomass and grain yield was measured immediately after harvesting and their dry weight were calculated after oven drying at 70 ^{0}C for 24 hours.

2.7 Economic analysis of biochar amendment

Cost-Benefit analysis of biochar farming applied at six different dosages for three subsequent year (year 2014 to year 2017) under maize and mustard-cropping system was performed on the basis of the agronomic results obtained. The agronomic cost included farm inputs (seeds, urea and manure) and labor for land preparation. Biochar production cost included labor for kiln construction and operation as well as health cost of gas (CO) and aerosol (smoke, $PM_{2.5}$) emissions during biochar making (analogous to Sparrevik et al. (2014)), in addition to climate cost of CH_4 emissions (taking into account the 27-fold higher global warming potential of CH_4 as compared to CO_2) during biochar making (Smebye et al., 2017; Sparrevik et al., 2015). The gas emissions from a flame curtain kiln were used for the cost-benefit analysis, as this novel method is the one of choice in practice and far preferable over traditional kilns, due to low gas and aerosol emissions, as well as easy and quick operation (Cornelissen et al., 2016). However, financial cost of biochar making, agronomic effect of the resulting biochar's, as well as methane emissions, would have been similar for traditional and flame curtain kilns (Cornelissen et al., 2016). Only CO emissions and resulting health effects would have been higher for traditional kilns. Thus, the outcome of the cost-benefit analysis would have been almost the same for traditional kilns (gross margins being maximally 5% lower, with the same trends between C price scenarios and biochar dosages). Income was calculated from crop sale (both maize and mustard) and possible carbon sequestration benefits at various C prices. Details of all costing are in SI (Table S3 and S4). Gross margin/profit of biochar-inclusive farming (Total income - Total cost) was calculated as a function of biochar dosage, assuming a medium social cost of carbon, SSC, of US$42 per ton CO_2 (SCC at 3% discount rate and emitted in 2020) (EPA, 2013). In addition, various carbon prices were used, ranging from no carbon price (US$0 per ton CO_2), as would be the current situation, to current voluntary carbon market prices (US$6 per ton CO_2), and a high-impact SSC of US$147 per ton CO_2 (EPA, 2013).

2.8. Statistical analysis

Data were analyzed through R software version 3.2.2. Normality and homogenous variances of all data sets were tested with Shapiro-Wilk – and Levene's test, respectively. One factor fixed effect ANOVA model was used to assess the effect of biochar addition on soil properties and crop yield for all three-year harvest. Post hoc Tukey test (pair wise comparison at $P=0.05$) was performed to assess the least significant difference (LSD) between the treatment means. Paired t-test was used to assess the effect of biochar between three-year crop harvests grown in respective treatment plots. Linear regression model was used to identify the relationship between various soil physicochemical properties and crop yield (third year) at a given level of biochar addition.

3. Results

3.1 Effect of biochar addition on soil fertility

Changes in soil physical and chemical properties as a result of various levels of biochar addition are presented in Table 2. Soil pH_{CaCl2} was significantly increased (*P<0.001*) at all levels of biochar addition. Related to this, average Al/Ca ratio was significantly reduced upon biochar addition above 10 t ha^{-1}, from a relatively low value of 0.21 without biochar though. Average soil CEC was significantly increased upon 40 t ha^{-1} BC addition compared with control soil. Exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}) were significantly increased at all levels of biochar addition to this soil, however, base saturation was already high without biochar addition (74%).

Biochar additions showed significant effects (*P<0.001*) on plant available phosphorous (P-AL) which was increased by 92% and 440% upon 10 t ha^{-1} and 40 t ha^{-1} of biochar addition compared with control soil respectively (Table 2). Similarly, soil moisture retention at field capacity and plant available water were significantly increased upon 40 t ha^{-1} biochar addition compared to control soil.

Table 2

Soil properties at different level of biochar addition for soil samples taken after 5th season (2.5 year); mean ± sd, n = 3. Different letters within the column of each treatment represent significant differences between various treatments on soil properties following one factor ANOVA (post hoc-Tukey test, p = 0.05).

Soil	**Treatments**					
Properties	**0 t ha^{-1} BC (control)**	**5 t ha^{-1} BC**	**10 t ha^{-1} BC**	**15 t ha^{-1} BC**	**25 t ha^{-1} BC**	**40 t ha^{-1} BC**

pH_{H2O}	5.14 ± 0.02a	5.29 ± 0.01b	5.24 ± 0.02b	5.46 ± 0.01c	5.55 ± 0.02d	5.70 ± 0.03e
pH_{CaCl2}	4.62 ± 0.01a	4.74 ± 0.01b	4.73 ± 0.01b	4.92 ± 0.01c	4.94 ± 0.02c	5.11 ± 0.03d
CEC ($cmol_c$ kg^{-1})	6.40 ± 0.28a	6.45 ± 0.10a	6.68 ± 0.09a	7.02 ± 0.38a	7.18 ± 0.17a	8.38 ± 0.52b
BS[1] (%)	74.3 ± 4.5a	76.0 ± 5.2a	80.7 ± 2.4a	91.0 ± 2.6bc	92.7 ± 2.5bc	96.3 ± 0.6c
Ca^{2+} ($cmol_c$ kg^{-1})	3.22 ± 0.05a	3.54 ± 0.06b	3.73 ± 0.07c	4.47 ± 0.24d	4.61 ± 0.00e	5.74 ± 0.48f
Mg^{2+} ($cmol_c$ kg^{-1})	1.22 ± 0.04a	1.31 ± 0.00b	1.38 ± 0.00c	1.66 ± 0.00d	1.73 ± 0.00e	1.96 ± 0.04f
Na^{+} ($cmol_c$ kg^{-1})	0.03 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.02a	0.04 ± 0.02a	0.03 ± 0.00a	0.05 ± 0.01a
K^{+} ($cmol_c$ kg^{-1})	0.21 ± 0.00a	0.23 ± 0.01b	0.26 ± 0.02b	0.27 ± 0.01b	0.30 ± 0.00c	0.38 ± 0.01d
H^{+} ($cmol_c$ kg^{-1})	0.92 ± 0.34d	0.78 ± 0.05d	0.61 ± 0.21cd	0.36 ± 0.19bc	0.25 ± 0.17b	0.00 ± 0.00a
Al^{3+} ($cmol_c$ kg^{-1})	0.71 ± 0.02e	0.58 ± 0.01c	0.65 ± 0.01d	0.22 ± 0.01a	0.26 ± 0.01b	0.25 ± 0.01b
Al/Ca (molar ratio)	0.21 ± 0.01c	0.17 ± 0.01b	0.18 ± 0.01b	0.05 ± 0.00a	0.05 ± 0.01a	0.05 ± 0.01a
Ca/Al (molar ratio)	4.67 ± 0.15a	6.03 ± 0.15a	5.73 ± 0.06a	20.67 ± 1.53bc	17.97 ± 0.75b	23.30 ± 2.60c
Total OC %	1.81 ± 0.01a	1.82 ± 0.01a	2.01 ± 0.02b	2.42 ± 0.01c	2.65 ± 0.01d	4.99 ± 0.01e
Total N (%)	0.18 ± 0.01bc	0.15 ± 0.01a	0.16 ± 0.01ab	0.18 ± 0.01bc	0.17 ± 0.01bc	0.20 ± 0.01c
Total H (%)	0.48 ± 0.01a	0.50 ± 0.01ab	0.51 ± 0.00ab	0.49 ± 0.01a	0.52 ± 0.01b	0.55 ± 0.01c
Available P (mg kg^{-1})	12.5 ± 0.6a	-	23.3 ± 0.6 b	-	-	65.3 ± 0.8 c
FC[2] (% vol.)	29.8 ± 1.8a	-	29.9 ± 1.3a	-	-	35.0 ± 0.7b
Wilting point (% vol.)	9.0 ± 0.15a	-	8.78 ± 0.64ab	-	-	9.75 ± 0.39b
PAW[3] (% vol.)	20.82 ± 1.97a	-	21.18 ± 0.78a	-	-	25.55 ± 0.54b

[1]Base saturation, [2]Field capacity and [3]Plant available water

3.2 Effect of biochar addition on soil carbon

Biochar addition showed significant effect on SOC at all levels of biochar addition except the lowest dosage (5 t ha^{-1} BC). Addition of manure containing 30% C to the soil with a C% of 1.6% should have resulted in 1.9% C in the control soil. Addition of a material with 70% C (biochar) and one with 30% C (manure) to soil containing 1.6% C (Table 1) in the five treatment proportions i.e. 5 t ha^{-1} BC (0.25%), 10 t ha^{-1} BC (0.5 %), 15 t ha^{-1} (0.75%) BC, 25 t ha^{-1} BC (1.25%) and 40 t ha^{-1} BC (2%) should have resulted in around 2.01 % SOC, 2.25 % SOC, 2.41 % SOC, 2.66 % SOC and 3.30 % SOC respectively. These values were close to the measured values of 1.82 %, 2.01 %, 2.42 %, 2.65 % and

4.99 % SOC respectively (Table 2). Thus, biochar addition mainly showed an additive effect on SOC over time in this soil.

BPCA analysis provides information on the proportion of condensed aromatic C in soil, and serves as a measure of pyrogenic C. On the basis of the calibration that around 24% of condensed C is converted into benzenepentacarboxylic (B5CA) and benzenehexacarboxylic (B6CA) during the nitric acid oxidation (Bostick, in revision), we calculated amounts of 91.6% condensed carbon in the pure biochar, 0.50 % in the non-amended soil, and 3.7 % in the soil amended with 40 t ha^{-1} biochar after 2.5 y (Table 3). Thus, 3.7 – 0.5 = 3.2% of the condensed C can be attributed to the added biochar, similar to the amount of biochar C originally added to the soil (4.99 – 1.81 = 3.18%, Table 2). This suggests that almost all condensed C in the biochar survived after five seasons (2.5 y) of aging under field conditions. The degree of aromatic condensation of the original carbon in the sample can be represented by the ratio of B5CA/BC6A (Schneider et al., 2010), as B5CA are formed from less condensed components than B6CA compounds . Thus, the 0.53 B5CA/B6CA ratio of biochar in the aged soil indicates it was less condensed, and perhaps more oxidized, than the fresh biochar with a B5CA/B6CA ratio of 0.35 (Table 3).

Table 3

BPCA composition of pristine biochar, aged biochar in the 40 t ha^{-1} plots (after 5 seasons) and the control soil.

Treatments	B5CA[1]	B6CA[1]	Pyrogenic C[2]	B5CA/B6CA
	mg BPCA per g soil		%	
Fresh biochar	57.3	163.5	91.6	0.35
Control soil	0.53 ± 0.03	0.68 ± 0.06	0.5	0.78
40 t ha^{-1} aged biochar	3.1 ± 0.3	5.8 ± 0.4	3.7	0.53

Notes:

[1] Benzenepentacarboxylic (B5CA) and benzenehexacarboxylic (B6CA) acids

[2] (B5CA + B6CA)*4.1/10

3.3 Crop yield

In the first year harvest, biochar addition did not show a significant effect (*p > 0.05*) on maize (Fig.1a) and mustard grain yield (Fig.1b) but significant effects (*p < 0.01*) on biomass of both crops were observed (Fig.S1). Significant effects of biochar addition on both crops' grain yield were observed during the second year's harvest (Fig.1, Table S1). Maize grain yield of the third season harvest (second year) increased by 50% (5.3 ± 0.4 t ha^{-1}), 47% (5.2 ± 0.5 t ha^{-1}) and 93% (6.8 ± 0.6 t ha^{-1}) at 15 t ha^{-1} BC, 25 t ha^{-1} BC and 40 t ha^{-1} BC addition respectively compared to control soil (3.5 ± 0.2 t ha^{-1}) (Fig.1a). Similarly, mustard grain yield of the fourth season harvest (second year) was increased by 96% (1.02 ± 0.14), 128% (1.19 ± 0.18 t ha^{-1}) and 134% (1.22 ± 0.16 t ha^{-1}) at 15 t ha^{-1} BC, 25 t ha^{-1} BC and 40 t ha^{-1} BC addition respectively compared to control (0.52 ± 0.01 t ha^{-1}) (Fig.1b). In addition to these biochar treatments (15 t ha^{-1} BC, 25 t ha^{-1} BC and 40 t ha^{-1} BC amendment), biochar addition at 10 t ha^{-1} BC also showed significant effect on mustard biomass production during the fourth season (Fig.S1b). Similar significant trends as observed for the second year harvest were observed for both maize and mustard crop yield (Fig.1, Table S1) and biomass production (Fig.S1, Table S2) during seasons 5 and 6 (third year harvest).

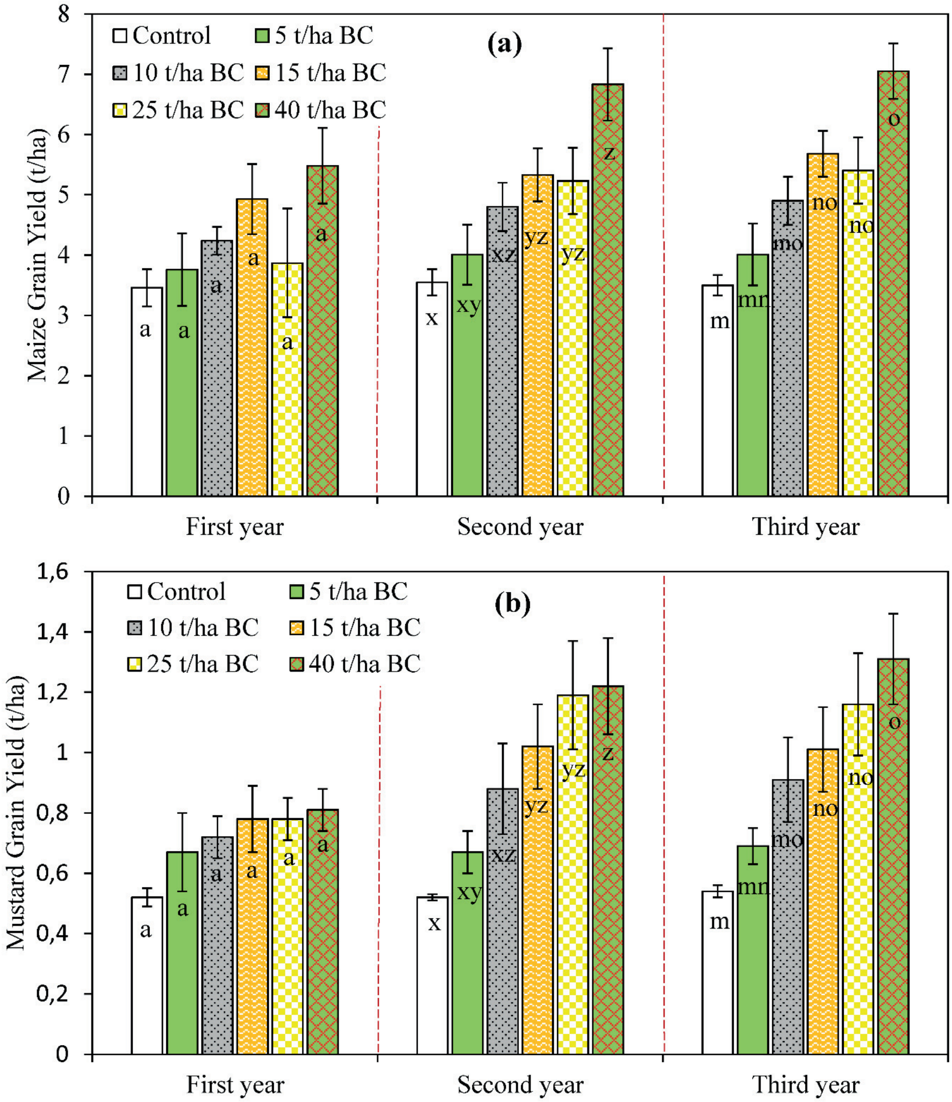

Fig.1. Effect of biochar addition on grain yield of maize (*fig a*) and mustard (*fig b*) over a period of three cropping years; mean ± SE, n = 4. Different letters inside a bar of each treatment represents significant differences between various treatments in a respective year following one factor ANOVA (post hoc-Tukey test, p = 0.05).

3.4. Cost-Benefit analysis through agronomic trials

Gross margin per ha cropped land was observed highest (4500 US$) for 15 t ha^{-1} biochar addition, when calculated based on the medium social cost of CO_2 price (42 US$ per ton) (Fig.2c), taking into account the CH_4 emission cost during biochar production and income/benefit when burying it in soil (C sequestration) in the respective biochar addition plot. Without a carbon price, gross margin still peaked at a biochar dosage of 15 t ha^{-1}, but at a lower value of around 3500 US$, and showing a sharper decrease with increasing biochar dosage above 15 t ha^{-1}. All numerical data can be found in the SI (Tables S3 and S4). Biochar produced from freely available *Eupatorium* through flame curtain soil pit kiln cost around 144 US$ per ton biochar (39US$ per ton CO_2-e.; including labor, packaging, storage and transportation) (Table S3). During biochar production, health cost (acute respiratory and chronic obstructive pulmonary diseases) was also considered, and amounted to US$ 1016 for the production of 40 t ha^{-1} biochar and US$ 381 for 15 t ha^{-1} biochar (Table S4). For comparison, C emission cost (in terms of CH_4) was as high as US$ 834 for 15 t ha^{-1} biochar.

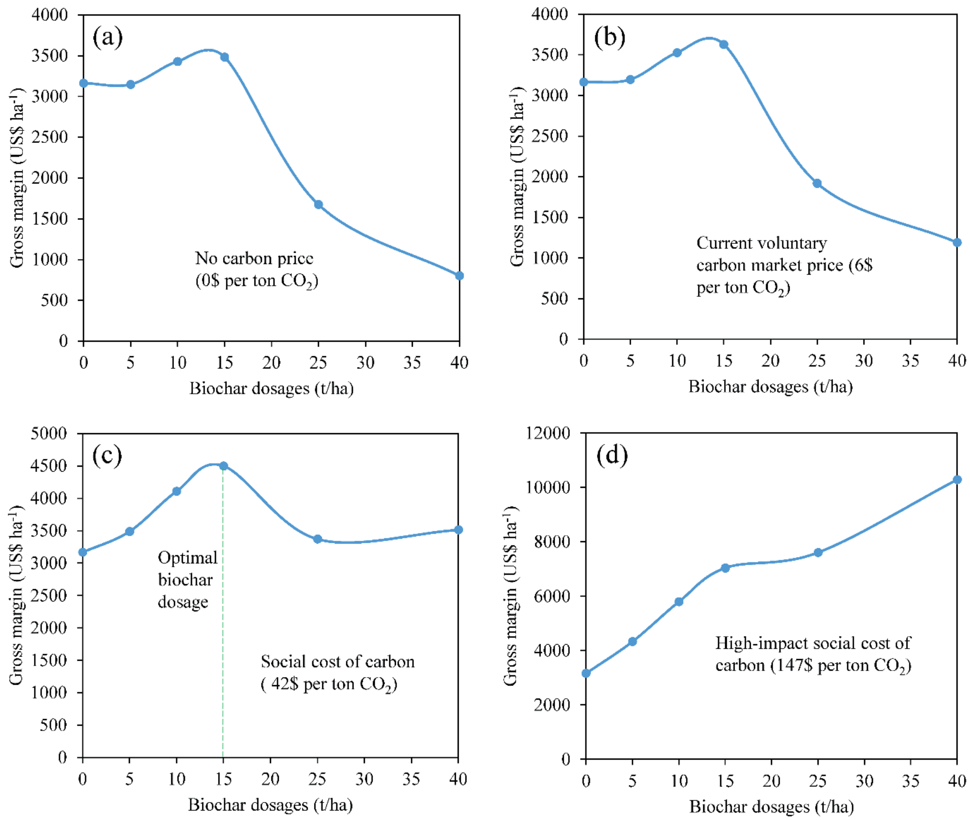

Fig.2. Gross margin of single biochar application at different levels, with varying carbon prices; a) no carbon price, b) voluntary market price (6$ per ton CO_2), c) medium social cost of C price (42$ per ton CO_2) and d) high-impact social cost of C price (147$ per ton CO_2) under maize and mustard cropping system over a three-year period.

4. Discussion

In this study, biochar addition showed a significant effect on grain yield of both maize and mustard of the second and third year's harvest, but not during the first year's harvest (Fig.1, Table S1). Both crop yields gradually increased with increasing biochar dosage over 10 t ha^{-1} (Fig.1, Table S1). In accordance with this, Major et al. (2010) reported increased maize yield in repeated years (after first year) with the amendment of only 20 t ha^{-1} biochar but not for a dosage of 8 t ha^{-1} during four year field trials (maize-soybean rotation) in Colombian savanna Oxisol. Similarly, another field study in Wales by Jones et al. (2012) reported significant effect of biochar on foliar N uptake and grass crop production only in second and third year harvest (not first year) at high biochar additions (25 t ha^{-1} and 50 t ha^{-1}) when applied in a Cambisol. The results indicate that biochar needs a certain level of aging in the soil in order to exert its positive yield effects. Haider et al. (2016) reported aged biochar to be more effective than fresh biochar in response to nutrient capture and delivery, which may lead to increased crop yield over time. This can possibly be explained by recent observations of the slow formation of an organic coating on biochar after aging in compost, increasing nutrient retention (Hagemann et al., 2017). In the experiment here, a similar phenomenon may have occurred over time in the presence of the repeatedly applied manure.

Biochar amendment (10 t ha^{-1} and 40 t ha^{-1}) significantly increased soil available P (P-AL) (Table 2), which showed a significant positive relationship with both maize ($R^2 = 0.95$, Fig.3a) and mustard grain yield ($R^2 = 0.92$, Fig.S3a) in this soil. In our previous study with the same soil and crop (maize) but under greenhouse conditions, P-AL appeared to be one of the most important growth limiting factors, which was effectively alleviated upon biochar addition (increased P-AL from 11 mg kg^{-1} at control to 84 mg kg^{-1} at 2% w:w biochar addition) thereby increasing maize biomass production (Pandit et al., 2018). In the present study, P-AL increased from 12.5 to 65 mg kg^{-1} upon biochar addition (40 t ha^{-1}) (Table 2), reaching the value (50-70 mg kg^{-1}) that is required for better crop growth (Krogstad et al., 2008). Improved P availability in low fertility acidic soil upon biochar addition has been reported by Hale et al. (2013), which has shown positive effect on crop production in P-deficient soils (Asai et al., 2009). In addition, biochar amendment increased soil moisture at field capacity and PAW by 5 % compared with control soil (Table 2), but only at a high dosage of 40 t ha^{-1}, thus, moisture was not expected to be the main growth-limiting factor in this soil, in line with our greenhouse observations (Pandit et al., 2018). In the much drier climate of Zambia, Martinsen et al. (2014), reported positive effect of biochar addition (10 % vol.) on crop yield, where PAW increased from 18.2% to 22.3%.

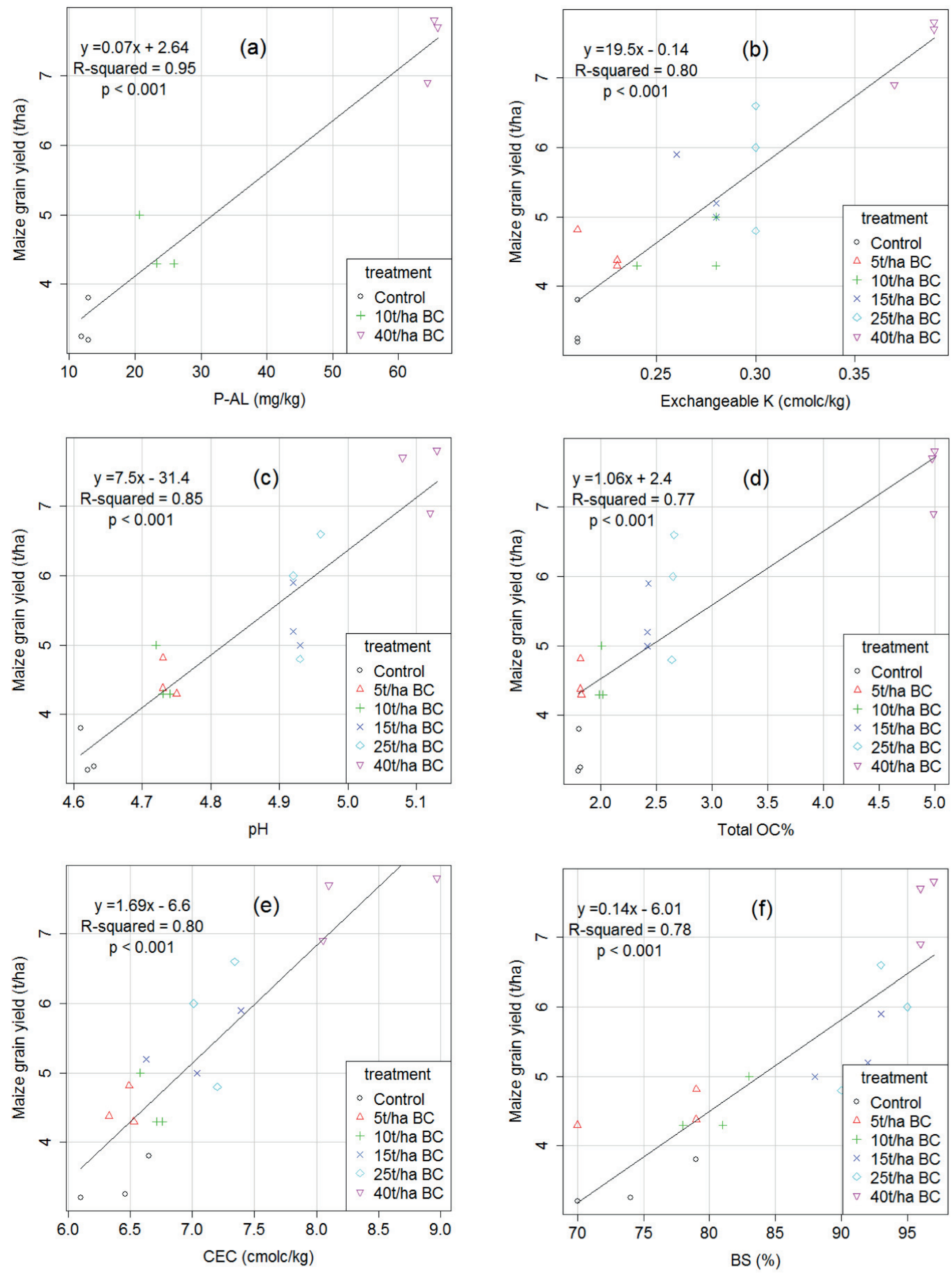

Fig.3. Relationship between soil parameters and maize grain yield (third year) as a function of biochar addition (n=18).

In addition, biochar amendment improved soil chemical properties such as soil pH, OC%, CEC and exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}) (Table 2). These measured soil chemical parameters were positively correlated with biochar doses (Fig.3). Biochar addition showed increased soil pH and reduced amount of Al^{3+} at all doses compared to the control (Table 2), but even the Al^{3+} content in the control treatment was below the levels where effects on plant roots can be expected (around 0.7 $cmol_c$ kg^{-1}) (De Wit et al., 2001), so we do not expect soil acidity alleviation to be the reason for the increased crop yields in the presence of biochar. In addition, soil organic carbon (SOC) was increased at all levels of biochar addition with the exception of 5 t ha^{-1} (Table 2), indicating the stability of C in the biochar over 2.5 years (Kuzyakov et al., 2014; Wang et al., 2016). On the basis of our data, it cannot be concluded whether the biochar was stable, or negative priming compensated for possible biochar decomposition. In multi-season field trials, Jones et al. (2012) reported increased soil pH upon biochar addition (50 t ha^{-1}) by 0.32 units and higher total SOC at the whole soil profile level over time due to very little effect of biochar on soil mineralization. On the other hand, long term field studies have shown gradual reduction of available exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+}) over time from the surface layer to lower horizon through leaching, however, these values were still higher than control soils (Jones et al., 2012; Major et al., 2010; Steiner et al., 2007). In our study, the measured soil exchangeable nutrients (Ca^{2+}, Mg^{2+} and K^{+}) sampled after the fifth season (2.5 y) from biochar amended soil were higher than those in control soil (Table 2), illustrating the effect of biochar on improved chemical soil fertility was sustained for at least 2-3 years in this soil.

Maize and mustard grain yield (5th and 6th seasons) were found to be positively correlated with various soil parameters upon biochar addition such as exchangeable K^{+} ($R^2 = 0.80$), pH ($R^2 = 0.85$), OC % ($R^2 = 0.77$), CEC ($R^2 = 0.80$) and BS% ($R^2 = 0.78$) (Fig.3, Fig S3). Related to K availability and its effect on crop yield, our previous mechanistic study (pot trial) in a similar soil revealed higher amounts of soil K upon biochar addition, which has shown significant positive relationship with maize growth (Pandit et al., 2018). Another field trial conducted by Gautam et al. (2017) in the similar silty loam soil from Rasuwa, reported increased K upon biochar addition (5 t ha^{-1}) which has shown beneficial effect on crop production. Similarly, positive effect of biochar addition (10 % vol.) on K availability and crop yield was observed in low productive tropical soils from Zambia (Martinsen et al., 2014). With respect to pH effect on crop yield, our previous study with a similar soil, showed that biochar addition increased soil pH, which led to a greater amount of available P, and contributed to increased crop yield (Pandit et al., 2018), showing a nutrient effect rather than liming effect of biochar. In our study, improved soil CEC and BS upon biochar addition illustrated the beneficial effect on crop yield (Fig.3), in line with many previous field studies carried out in low fertile acidic tropical soils (Cornelissen et al., 2013; Martinsen et al., 2014; Yamato et al., 2006). Soil inorganic N ($NO_3^-$and NH_4^+) was not considered in this study, as the amount of extractable $NO_3^-$and NH_4^+ did not reveal significant effect on maize biomass production in a similar soil under controlled greenhouse conditions (Pandit et al., 2018). Overall, positive crop yield effect upon biochar addition was possibly due to improved plant available nutrients (mainly P and K) in this soil.

Cost-benefit analysis

Currently there is no possibility for payment of C credits to farmers and thus, the price of CO_2 was set to zero in one of our scenarios. In this scenario, gross margin was observed to peak at 3481 US$ per ha (Fig.2a), however, the incentive for biochar use from the farmer's perspective is small as the difference in gross margin between no biochar amendment (3163 US$ over 3 y) and optimal biochar amendment rate (15 t/ha; 3481 US$ over 3 y) was only 10%. Gross margin was drastically reduced for higher biochar dosages (25 t $ha^{-1}$and 40 t ha^{-1}) under the zero CO_2 price regime as the increase in crop yield was not worth the investment of adding such high amounts of biochar. At a voluntary market C price of 6$ per ton CO_2 (Fig.2b) as well as at a medium social cost of CO_2 price of 42 US$ per ton (Fig.2c), gross margin also peaked at 15 t ha^{-1} biochar with the clearest incentive for making biochar at the 42US$ CO_2 price (gross margin for 15 t ha^{-1} biochar 4500 US$ ha^{-1} over 3 years, and for no biochar 3163 US$ ha^{-1}; a difference of 42%). At a high social cost CO_2 price (147$ per ton CO_2), gross margin continued to increase with biochar dosage, as theoretical income from such highly priced potential carbon credits would exceed that from crop yields (Fig.2d). Based on the significant effect of biochar applied at 15 t ha^{-1} on maize crop (Fig.1a) and mustard crop (Fig.1b) in a subsequent year along with higher gross margin, this study suggests the optimal biochar dosage under local farmers practices is 15 t ha^{-1}. It should be noted that this is true for this particular soil/biochar/farming system combination, and several multi-season field studies are needed to identify the long-term agronomic, financial and C sequestration benefits of biochar in Nepalese soil. A relatively high application rate of 15 t/ha could be surmountable for smallholder farmers because average land holding size in Nepal is small (around 0.7 ha, CBS, 2001/2002).

This is one of the first studies taking into account both the climate cost of methane emissions and the health cost of CO and aerosol emissions during biochar production. However, these are not costs that are directly felt by the farmer making the biochar. Thus, the direct farmer incentive to make biochar is actually higher than represented in the graphs in Fig. 2 for those cases where C price is higher than zero. For example, for biochar dosages of 0 and 15 t ha^{-1} at a medium social cost carbon price, the gross margin excluding these costs not directly felt by the farmer, was calculated to be 3163 US$ and 5712 US$ respectively (instead of 3163 and 4500 US$, respectively), a difference of 80% (Fig. S4).

5. Conclusion and recommendation

Significant effects of biochar addition on both maize and mustard grain yield were observed during second and third year harvests. Thus, long-term agronomic effects of a single application of biochar were observed. Biochar addition at 15 t ha^{-1} was found to be optimal, and this dosage is probably feasible from agronomical, economic and environmental perspectives, at least for the intensive cropping pattern in the mid hills of Nepal. Farmers can fetch a gross margin of around 4500 US$ per ha over 3 years (1500 US$ per ha per year) at a 15 t ha^{-1} biochar application, which was 42% more than that from no biochar amendment (3163 US$ per ha over 3 years i.e. 1054 US$ per ha per year). The average landholding size of hilly region, Nepal was 0.7 ha (CBS, 2001/2002), and thus, biochar application (15 t ha^{-1}) could increase the average margin per household by 3150 US$ over 3 years (1050 US$ per year) compared to control (2214 US$ over 3 years i.e. 738 US$ per ha per year).

Increased margin of 42% through biochar amendment would significantly improve the socio-economic status of poor farmers in Nepal where 25% of rural households are still living below the poverty line (average household income < 1000US$ per year, NLSS 2011). However, Nepal encompasses varied geography ranging from tropical (< 1000 msl) to high hills (> 7000 msl) having different soil type and crop yield per unit of land, which may have diverse crop yield effects resulting in varied socio-economic benefits of biochar amendment. Thus, further study is needed to explore the effectiveness of biochar in various topography, soil and crop types and assess their profitability considering both financial and carbon benefits. The present study provides an approach to obtain this information.

One ton of biochar can be produced from around five ton of dry Eupatorium (20% conversion efficiency) by the farmers themselves on their farmland. In Nepal, *Eupatorium adenophorum*, an invasive shrub regenerated naturally in forest, farm upland and riverbanks is found in abundant quantities, and can be considered a sustainable feedstock to produce good quality biochar at relatively low cost, thus turning a pest into a potential resource. Flame curtain pyrolysis kilns (Kon-Tiki) are the pyrolysis method of choice, with no material resource demand and low human resource demand for their construction, and their clean, easy and fast mode of operation (Cornelissen et al., 2016).

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Supplementary Information (SI) Paper

Multi-year double cropping biochar field trials in Nepal: finding the optimal dosage through agronomic trials and cost-benefit analysis

Naba Raj Pandit[a,b,c], Jan Mulder[b] , Sarah E.Hale[a], Andrew R. Zimmerman[d] , Bishnu Hari Pandit[e], Gerard Cornelissen[a,b]

[a] *Norwegian Geotechnical Institute (NGI), Oslo, Norway*

[b] *Faculty of Environmental Sciences and Natural Resource Management (MINA), Norwegian University of Life sciences (NMBU), Ås, Norway*

[c] *Nepal Agroforestry Foundation (NAF), Kathmandu, Nepal*

[d] *University of Florida, Department of Geological sciences, Gainesville, FL, USA*

[e] *Kathmandu Forestry College (KAFCOL), Kathmandu, Nepal*

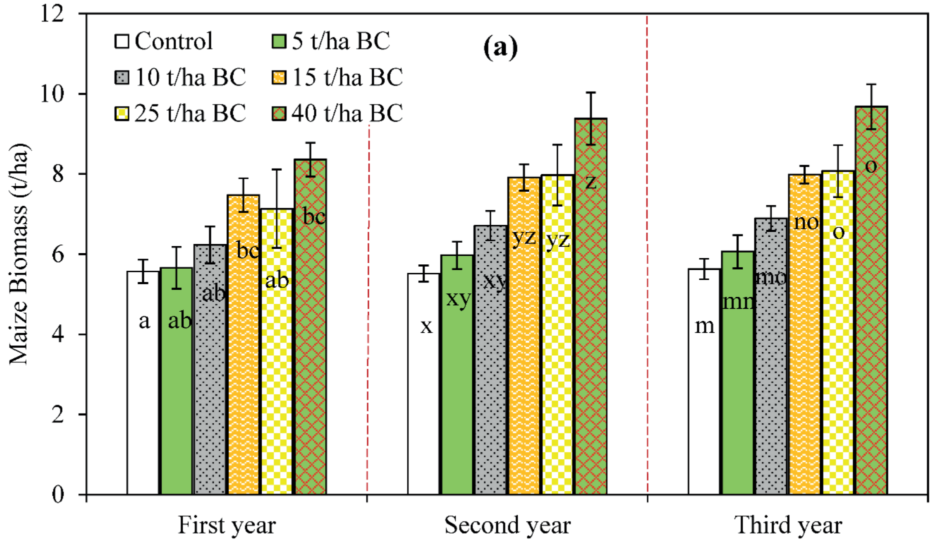
* Corresponding author. Email: navraj20@gmail.com

Images of Eupatorium feedstock, biochar production and application in Nepal

Image.S1. Eupatorium feedstocks growing in natural conditions in forest area (left), farm upland (middle) and bank of the river (right)

Image.S2. Burning Eupatroium in a pit (left) and biochar application in research field trials (right)

Fig.S1. Location of field trials, Rasuwa district, Nepal



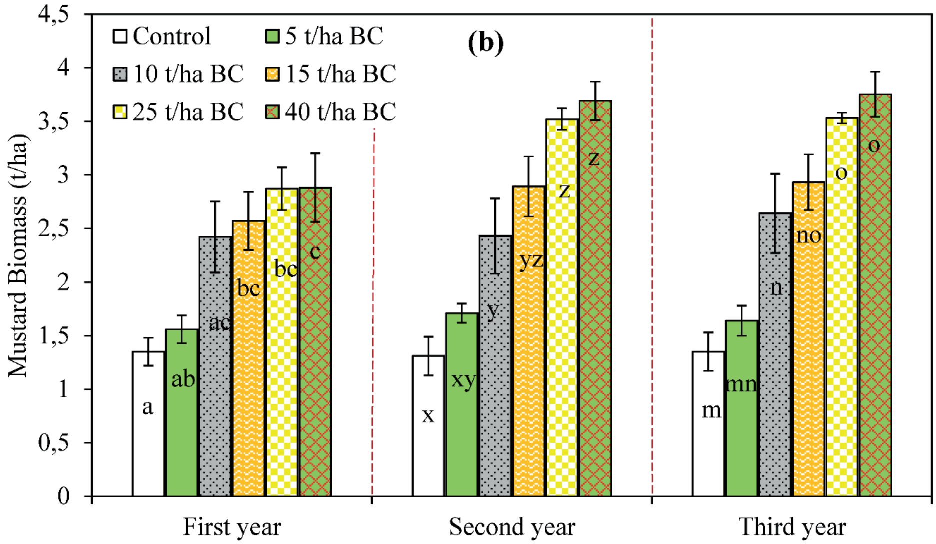


Fig.S2. Effect of biochar addition on biomass of maize (*fig a*) and mustard (*fig b*) crop over a period of three cropping years; mean ± SE, n = 4. Different letters inside a bar of each treatment represents significant differences between various treatments in a respective year following one factor ANOVA (post hoc-Tukey test, p = 0.05).

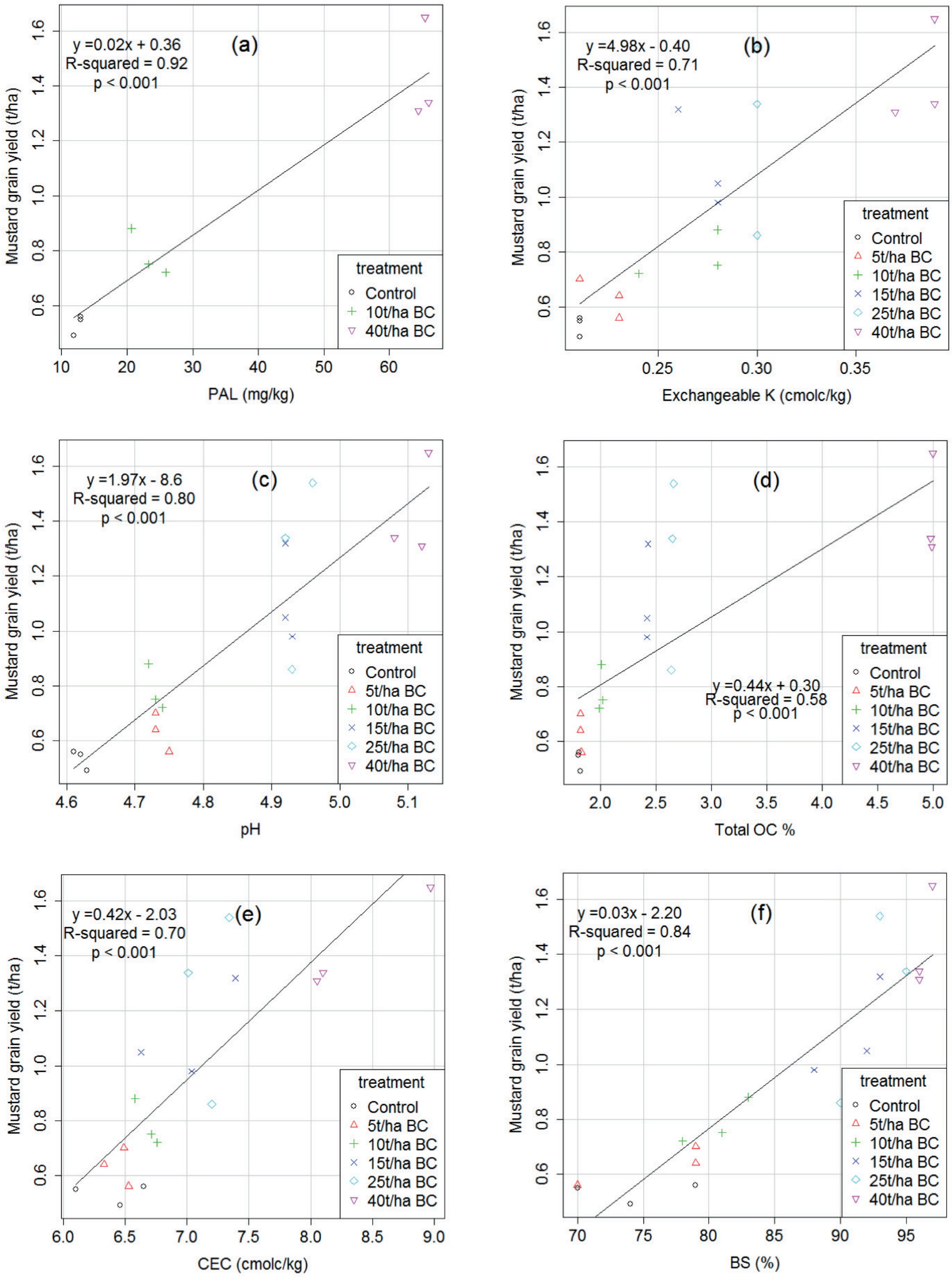


Fig.S3. Relationship between soil parameters and mustard grain yield as a function of biochar addition (n=18).

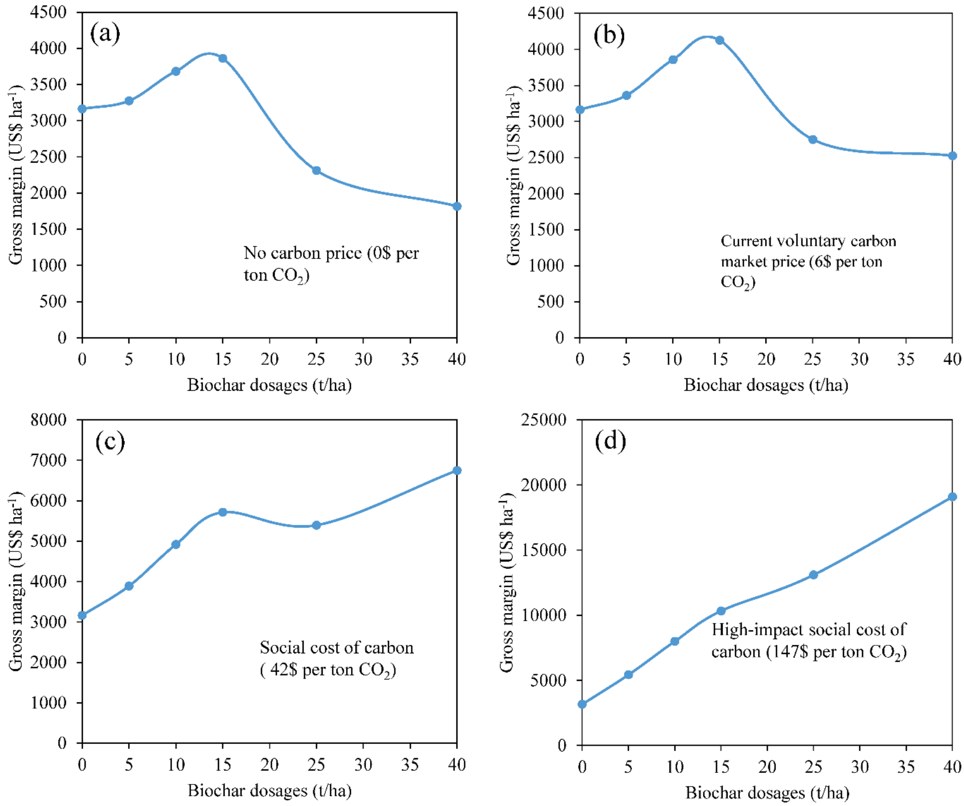


Fig.S4. Gross margin of single biochar application at different levels, with varying carbon prices without taking into an account health cost and C emission cost during biochar making: a) no carbon price, b) voluntary market price (6$ per ton CO_2), c) medium social cost of C price (42$ per ton CO_2), and d) high-impact social cost of C price (147$ per ton CO_2) under maize and mustard cropping system for three-year period.

Table S1

Effect of biochar treatments on maize and mustard grain yield (t ha^{-1}) between three cropping year. Value inside the table are the p-value along with their significant codes (n.s > 0.05., *<0.05 and **< 0.01) via paired t-test between two groups, n=4, ns denotes non-significant.

Treatments	Maize grain yield (t/ha)			Mustard grain yield (t/ha)		
	1st yr. & 2nd yr.	1st yr. & 3rd yr.	2nd yr. & 3rd yr.	1st yr. & 2nd yr.	1st yr. & 3rd yr.	2nd yr. & 3rd yr.
0 t ha^{-1} BC	0.80 ns	0.81 ns	0.45 ns	0.75 ns	0.43 ns	0.23 ns
5 t ha^{-1} BC	0.10 ns	0.09 ns	0.88 ns	0.98 ns	0.84 ns	0.36 ns
10 t ha^{-1} BC	0.02*	0.02*	0.25 ns	0.13 ns	0.06 ns	0.24 ns
15 t ha^{-1} BC	0.03*	0.05*	0.24 ns	0.02*	0.02 *	0.76 ns
25 t ha^{-1} BC	0.04*	0.02*	0.21 ns	0.05*	0.05*	0.21 ns
40 t ha^{-1} BC	0.003**	0.007**	0.78 ns	0.02*	0.009**	0.02 ns

Table S2

Effect of biochar treatments on maize and mustard biomass production between three cropping year. Value inside the table are the p-value along with their significant codes (n.s > 0.05., *<0.05 and **< 0.01) via paired t-test, n=4.

Treatments	Maize biomass (t/ha)			Mustard biomass (t/ha)		
	1st yr. & 2nd yr.	1st yr. & 3rd yr.	2nd yr. & 3rd yr.	1st yr. & 2nd yr.	1st yr. & 3rd yr.	2nd yr. & 3rd yr.
0 t ha^{-1} BC	0.67 ns	0.42 ns	0.32 ns	0.62 ns	1.0 ns	0.48 ns
5 t ha^{-1} BC	0.35 ns	0.14 ns	0.40 ns	0.29 ns	0.6 ns	0.39 ns
10 t ha^{-1} BC	0.05*	0.02*	0.19 ns	0.73 ns	0.08 ns	0.03 ns
15 t ha^{-1} BC	0.03*	0.05*	0.57 ns	0.008**	0.01*	0.53 ns
25 t ha^{-1} BC	0.04*	0.05*	0.92 ns	0.03*	0.04*	0.92 ns
40 t ha^{-1} BC	0.03*	0.008**	0.13 ns	0.01*	0.01*	0.40 ns

Table S3

Cost of per ton biochar production from Euaptorium feedstock in Rasuwa district, Nepal

S.No	Input	Unit	Qty	Unit cost (US$)	Amount (US$)	Remarks
1	**Biochar production cost (1 ton)**					
1.1	Eupatorium feedstock	Ton	1	Free	Free	Freely available in forest, farm upland and bank of river
1.2	Labor required to chop and collect 1 ton Eupatorium	p/d[1]	4	4	16	1 ton eupatorium produce 200 kg dry biochar (20% biochar yield)
1.3	Labor wage to chop and collect 5 ton Eupatorium	Ton	5	16	80	5 ton Eupatorium required to produce 1 ton biochar (20% biochar yield)
1.4	Cost of flame curtain soil pit kiln	kiln	1	4	4	Only labor charge @ US$ 4 to build 1 m^3 soil pit kiln[2]
1.5	Labor wage for biochar production	Ton	1	25	25	200kg biochar per day[2] @ 500 NRs; 1 ton biochar with 2500 NRs in 5d
1.6	Packaging and storage	Ton	1	10	10	2 p/d required to perform packaging of 1 ton biochar
1.7	Transportation of biochar to farm	Trip	1	25	25	Transportation in vehicle (lorry) covering distant not more than 1 hr
Total cost of production (1 ton biochar)					***144***	

[1] p/d represent person per day working 8 hours per day

[2] 1m^3 flame curtain pyrolysis soil pit kiln have the capacity to pyrolyze 200 kg dry Eupatorium, which can produce around 40 kg biochar in each run. One farmer or person can execute 5 run per day in such kiln (wage labor 500 NRs per day), which can produce 200kg biochar in total per day.

Table S4

Economical analysis of biochar application in agricultural system (three-year sequential maize and mustard plantation and harvest)

S.no		Biochar dosages (ton per hectare)						Remarks
	Description	Control	5t/ha	10t/ha	15t/ha	25t/ha	40t/ha	
1	**PRODUCTION COST**	Cost in US$						
1.1.	**Financial cost**							
	Biochar	0	720	1440	2160	3600	5760	From Table 1
	Seeds[1]	40	40	40	40	40	40	
	Urea (60kg N/ha) [2]	270	270	270	270	270	270	0.6 US$ per kg Urea
	Farm yard manure[3]	360	360	360	360	360	360	
	Labour[4]	900	900	900	900	900	900	
1.2.	**Health cost (per household)[5]**							
	Acute Respiratory Infection (ARI) morbidity		4.9	9.9	14.8	24.7	39.6	Per ton CO_2; 0.99 US$
	Acute Respiratory Infection (ARI) mortality		25.3	50.6	75.9	126.5	202.4	Per ton CO_2; 5.06 US$
	Cost chronic obstructive pulmonary diseases (morbidity)		10.3	20.6	30.9	51.5	82.4	Per ton CO_2; 2.06 US$
	Cost chronic obstructive pulmonary disease (mortality)		86.5	173	259.5	432.5	692	Per ton CO_2; 17.3 US$
1.3	**Carbon cost (**CH4 emission per ton biochar produced)[6]							
	Medium social cost C price ($42 per ton CO_2)		278	556	834	1390	2224	($55.6 per ton biochar production
2	**INCOME**	Income in US$						
2.1	**Income from crop sale**							
	Maize grain yield	3153	3534	4182	4782	4350	5808	Maize and mustard sale from three year harvest
	Mustard grain yield	1580	2030	2510	2810	3130	3340	
2.2	**Income through carbon (C) sequestration**							80% C stability in biochar
	No carbon price (CDM market; $0 per ton CO_2)	0	0	0	0	0	0	
	Voluntary carbon market price; $6 per ton CO_2)		88,1	176.2	264.2	440.4	704.6	CO_2-C = 3.67*CO_2
	Medium social cost C price ($42 per ton CO_2)		616.6	1233.1	1849.6	3082.8	4932.5	
	High-impact social C price ($147 per ton CO_2)		2157.9	4315.9	6473.8	10789.8	17263.6	
2.3	**GROSS MARGIN[7]**	Gross margin in US$						
	No carbon price (CDM market; $0 per ton CO_2)	3163	3147	3428	3481	1675	802	
	Voluntary carbon market price; $6 per ton CO_2)	3163	3196	3525	3627	1918	1190	
	Medium social cost C price ($42 per ton CO_2)	3163	3486	4105	4497	3368	3510	
	High-impact social C price ($147 per ton CO_2)	3163	4333	5800	7039	7605	10289	

[1]35 kg maize seeds (0.8 US$ per kg) required for three years per hectare; For mustard, 12 kg seeds per hectare at the rate of 1 US$ per kg.

[2]60 kg N per ha i.e. 150 kg Urea per ha (46% N in Urea); per kg urea cost US$ 0.6; for three years 150 kg X 0.6 X 3 (US$ 27). Urea was applied only for maize crop based on farmers practice

[3]20 t /ha FYM i.e. 20000 kg FYM, one bamboo basket (doko) consists of 50kg FYM and cost Rs 0.3 US$. Thus, 120 US$ for 400 doko per year for maize; 360 US$ for three year. FYM was applied only for maize crop based on farmers practice.

[4] Land preparation/sowing (180 US$/ha/yr) and weeding (120 US$/ha/yr, 30 person @ 4 US$/person/ha), thus, 900 US$ in three year period for maize and mustard cropping system.

[5] Health cost was taken from Indonesia during biochar making, which is similar in the context of Nepal

[6]1 ton BC equivalent to 1,35 ton CO_2 (CH_4 27 times higher than CO_2) . 0.049 ton CH_4 per ton biochar.

[7]Calculation of gross margin (Income – production cost) at all level of C sequestration for eg. Gross margin for Marginal climate change cost ($42 per ton CO_2) = Income from crop sale + C sequestration benefit (($42 per ton CO_2) – Production cost.

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

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