Norwegian University of Life Sciences

Preface

This Master's thesis is the result of an experimental study conducted with support from the Department of Ecology and Natural Resource Management at the Norwegian University of Life Sciences (UMB).

I would like to thank to my supervisor, Professor Mikael Ohlson, Norwegian University of Life Sciences, and co-supervisor, Assistant Professor Michael J. Gundale, Swedish University of Agricultural Sciences. They were very helpful, gave me patient guidance and advisory through the whole project and most importantly granted me the opportunity to participate on this interesting research.

I want to thank to Doctoral student Nathalie Pluchon from the Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, for helping me collecting soil samples. I am also grateful to the Centre for Plant Research in Controlled Climate (SKP) at the Norwegian University of Life Sciences, for letting me use their properties and equipment. My special thanks go to M.S. Yevgenia Tomkiv, M.S. Padina Kouhrani Sefiddashti and B.C. Anne Guri Weihe for their help and support during the greenhouse experiment and in the laboratory.

Finally I would like to thank my friends and family for their help and support during this project.

Abstract

The objective of this study is to use plant phytometers to test whether biochar amendment increases soil fertility. The phytometric method has the asset of measuring the actual effect of biochar treatment on changes in plant productivity instead of investigating biochar properties and its effect on soil. As plant phytometers were selected two species with contrasting life-histories. (1) *Betula pendula*, which is a long-lived and relatively slow-growing perennial specie and (2) *Phacelia tanacetifolia*, an annual herbaceous and relatively fast-growing specie. Soil was collected in fairly homogenous ecosystems from 5 different biogeographic regions (i.e. alpine, arctic, boreal, mediterranean and temperate). The biochar was prepared mainly from *Pinus sylvestris* (90 %), under 450 - 500°C charring temperature.

Plant productivity differed significantly among the soils as expected. The productivity was highest in the temperate soil and lowest in the boreal soil. However, only the temperate soil seemed to be highly susceptible to the biochar treatment. Although there were various significant results of the biochar treatment, there was no clear and consistent difference between the pots augmented with biochar and the control pots without biochar. Actually, plant productivity decreased as a result of biochar addition in some cases, which was unexpected. This suggests that the used biochar may have had a toxic impact on the phytometer plants. The results of this study show the need for further systematic research and development of a minimum standard characterization criteria of biochar used as a soil amendment.

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1. Introduction

Wildfire through lightning strike is a principal natural disturbance regime in a broad range of terrestrial ecosystems (Zackrisson, 1977; Preston, 2009). It not only releases carbon dioxide but also converts approximately 1-3% of burning plant biomass into pyrogenic carbon (Preston, 2009), which is known to have rejuvenating effects on forest soil properties and to improve plant growth (Wardle et al., 1998; Lehmann et al., 2011). Although information about fire induced changes in soil is available from grasslands and agricultural areas in temperate and tropical regions (Preston and Schmidt, 2006), and also from boreal forests (Arocena and Opio, 2003; Pietikäinen, 1999), there are almost no reports that deal with other biogeographic regions, such as the arctic and alpine. In general, the mechanisms responsible for the soil rejuvenating effects are inadequately explained and the ecological impact of pyrogenic carbon is poorly understood (Zackrisson et al., 1996; Lehmann et al., 2011). However pyrogenic carbon, including charcoal and other forms of black carbon, is known for its resistance to oxidation and microbial decay. Hence, it has been suggested as a possible long term carbon sink (Preston, 2009; Preston and Schmidt, 2006; Harden et al., 2000).

Recent studies have suggested that black carbon represents a significant soil organic carbon pool (Ohlson et al., 2009; Masiello, 2004; Schmidt and Noack, 2000). Since soil organic carbon is the largest carbon stock of the global terrestrial carbon reservoir (Verma et al., 2010), even a small change in it might have a consequential impact on the global carbon balance and hence on the global climate (IPCC, 2007). Lehmann (2007a) suggests biochar (which is, by definition, charcoal used as a soil amendment (Lehmann and Joseph, 2009)) as a possible apparatus for relevant carbon sequestration. Therefore pyrolytical biochar production and its subsequent application into soils is considered in long term carbon sequestration strategies and globally evaluated as one of the means to mitigate climate change and improve soil fertility (Khodadad et al., 2010; Lehmann et al., 2011).

Although the recalcitrant properties of biochar (Gundale and DeLuca, 2006; Nguyen et al., 2008) with its $10^3 – 10^7$ years of carbon half-life (Zimmerman, 2010), indicate its usefulness as a potential long-term carbon sink (Goldberg, 1985; Cheng at al., 2008; Lehmann, 2007a; Nguyen et al., 2008), biochar remains biologically active and so, might have major effects on soil biological processes (Zackrisson et al., 1996; Lehmann et al., 2011). Investigations have been made in order to assess wildfire production of charcoal (Ohlson and Tryterud, 1999) and the influence of

biochar addition on soil properties (Nguyen et al., 2008), microbial activity (Khodadad et al., 2010, Lehmann et al., 2011; Kim et al., 2007), nutrient uptake (Wardle et al., 1998) and hence on plant growth and productivity (Warnock et al., 2007; Wardle et al., 1998; Kishimoto and Sugiura, 1985). Despite all this, the molecular change mechanisms in biochar, which influence nutrient cycling and microbial activity are largely unknown (Nguyen et al, 2008).

Even with our lack of scientific understanding, biochar use in agriculture has been documented in ancient civilizations around the world. Augmented soils have been reported in the Americas, Asia and Africa (Elad et al., 2011). For example, even after hundreds of years, Terra Preta de Indio (Amazonian Dark Earth), one of the best documented ancient carbon-enhanced areas in the world (Sombroek, 1966), is fertile and shows a high accumulation of stable organic matter (Somobroek, 1966; Lehmann et al., 2003). This is accounted to the increase in nutrient holding capacity after biochar amendment (Smith, 1980). Another effect observed in Terra Preta, and attributed to the unique structure of biochar (Liang et al., 2010), is the greater level of microbial diversity and activity (Kim et al., 2007; Jesus et al., 2009; O'Neill et al., 2009). Furthermore, improved plant response after biochar amendment can be explained by the nutrient content and by several indirect effects (Kolton et al., 2011) such as: increased water and nutrient retention (Novak et al., 2009; Elad et al., 2011; Chan et al. 2007), pH rise in acidic soils (Novak et al., 2009; van Zwieten et al., 2010), neutralization of phytotoxic compounds (Wardle et al., 1998; Gundale and DeLuca, 2006), promotion of mycorrhizal fungi (Warnock et al., 2007), alteration of soil microbial populations due to the special structure of biochar (Kim et al., 2007; Steiner et al., 2008; Zackrisson et al., 1996; Pietikäinen, 1999), induction of systematic resistance to several phytopatogens (Elad et al., 2010) and impact of biochar on various other soil processes mainly through selective sorption and cation exchange capacity (Preston and Schmidt, 2006; Khodadad et al., 2010; Novak et al., 2009; Zackrisson et al., 1996).

Biochar is suggested to positively influence soil fertility and microbial composition, biomass and activity. However, the final impact may be influenced by several factors, such as: fundamental soil properties (Lehmann et al., 2011), amount of biochar incorporated into soil (Glaser et al. 2001) and attributes of biochar (chemical and physical) which are given as a function of feedstock and pyrolytical conditions (Zimmerman, 2010). On the micro- and nanometer scale biochar particles appear as a disordered mixture of C clusters and mineral elements (i.e. ash inclusions) with large internal surface areas and pores (Lehmann et al., 2011). Fungi as well as bacteria have been suggested to have better protection against predators, grazers, and competitors when inhabiting biochar structures (Thies and Rillig, 2009; Ogawa, 1994; Lehmann and Rondon, 2006). The

source material (plant species and plant tissue) may influence the total surface area, pore size distribution and hence biochar functionality (Warnock et al., 2007; Keech, 2005; Downie, 2009). For example, source material with large diameter cells can lead to greater quantities of macropores in biochar particles (Lehmann et al., 2011; Gundale and DeLuca, 2006), which might enhance adsorption of phenolic compounds (Keech et al. 2005). Subsequently, sorption of organic (Kasozi et al., 2010; Joseph et al., 2010) and mineral materials (Liang et al., 2006; Pignatello et al., 2006) from the soil can cause pore silting (Kwon and Pignatello 2005), which may lead to changes in surface area and pore volume (Lehmann et al., 2011). However, the source material and soil properties are not the only important factors controlling pore size distribution.

Another highly important factor is charring temperature (Gundale and DeLuca, 2006; Warnock et al., 2007). With higher temperatures biochar is more likely to have finer pores (Warnock et al., 2007), which (when diameter <20 µm) allow entry of bacteria, fungi and microbe feeding nematodes but not microarthropods (Zackrisson et al., 1996). The pore space and energy available to soil biota is thereby influenced by sorption, which in turn may be influenced by source material, charring temperature and soil properties (Lehmann et al., 2011). Additionally, the total microbial abundance might be influenced by the variety of living conditions caused by the liming effect and pH of fresh biochar (Lehmann et al., 2011). Feedstock and charring temperature have the main impact on the pH level of fresh biochar, which can vary from below 4 to above 12 (Lehmann, 2007b).

Lastly, pore size distribution determines whether biochar can retain moisture within its structure and hence allow continued hydration of microorganisms in drying soil (Lehmann et al., 2011). Considering its complex structure, the biochar particle can be compared to a soil aggregate (Zackrisson et al., 1996), which may provide to some extent such functions as a habitat for soil biota, organic matter protection, and retention of soil moisture and nutrients (Lehmann et al., 2011).

Lehmann (2007b) suggests combining pyrolytical energy production and biochar soil amendment. Combustion of organic matter and application of biochar to the soils would, in this case, mean a win-win situation. On one hand, it would be a tool for diversification of renewable energy supplies by turning organic waste into a source of energy, while mitigating global climate changes by active carbon sequestration from the atmosphere (Elad et al., 2011). On the other hand, it would also be an apparatus for intensification of sustainable food production by increasing net primary productivity and degraded lands regeneration (Lehmann, 2007b).

However, evidence is lacking on whether biochar amendment will have positive effects across all biogeographic systems and hence if it can be used worldwide. Therefore, in this study I investigate how biochar affects plant growth on soils from different biogeographic regions under controlled environmental conditions. I hypothesise that (i) there is a significant difference between soils from different biogeographic regions, (ii) the biochar treatment has a general and positive effect on plant production across all biogeographic regions and (iii) the effect of biochar is weaker in fertile soils than in low nutrient soils, expressed by a significant soil x treatment interaction effect.

2. Materials and methods

2.1 Soil origin

Soil was collected from 5 different biogeographic regions. In each region the soil samples were collected from six (10x10 m) plots. The plots were from a fairly homogenous ecosystem in the given region. Samples were taken from 5 positions in each plot by a soil sampling steel cylinder with a diameter of 10 cm. Each soil sample had a volume of approximately 1 litre. The collected soil samples were stored in the dark, at +4°C temperature, for about 3 months before they were used in the greenhouse experiment.

Arctic soil was collected on the island of Vardøhuus, Finnmark County, 10 m above the sea level, in the North-East Norway (70°22'N; 31°6'E). The site is characterized by low-angle light and a short growth season due to cold temperatures in winter and limited summer conditions. The predominant vegetation is tundra heath of the Arctic Empetrum-Dicranum-Lichen type (Oksanen and Virtanen, 1995). This vegetation is poor in species and the characteristic plants in the area where I collected the soil are: *Rubus chamaemorus, Eriphorum vaginatum, Empetrum hermaphroditum, Betula nana,* and *Dicranum scoparium.*

Alpine soil was collected close to Båtskaret, 1140 m above sea level, in the South-Eastern part of the mountain area Jotunheimen, Oppland County, central Norway (61°96'N; 08°86'E). This site represents typical nutrient poor and acidic alpine heath vegetation. The following vascular plant species and lichens are characteristic to the site: *Empetrum hermaphroditum, Loiseleuria procumbens, Phyllodoce caerulea, Solidago virgaurea, Betula nana, Salix herbacea, Stereocaulon paschale, Cladonia rangiferina,* and *Cetraria islandica*.

Boreal soil was collected from nearby Mosjøen Lake, 281 m above sea level, in the Southern part of Østmarka, Oslo County, western Norway (59°49'N; 11°00'E). This site was selected to represent the Norwegian spruce - bilberry forest type, which is a wide-spread forest type all-over boreal Fennoscandia (Seppä et al., 2009). To provide soil from sites with typical Norwegian boreal forest vegetation, sites with predominance of *Polytrichum commune* were omitted. Typical plant species are: *Vaccinium myrtillus, Maianthemum bifolium, Deshampsia flexuosa, Pleurozium schreberi,* and *Hylocomium splendens.*

Temperate soil was collected from an ash-elm forest located 130 m above sea level, 2 km west of the village Degeberga, Skåne County, South-Eastern Sweden (55°83'N; 14°05'E). The site is characterized by moist and nutrient rich conditions. Characteristic herbaceous species include: *Allium ursinium, Dentaria bulbifera, Stellaria nemorum, Mercurialis perenni, Lamiastrum galeobdolon* and *Aegopodium podagraria.*

Mediterranean soil was collected from a maple-oak forest in Western France (46° 10N'; -0° 22'W), 60 m above the sea level, 25 km south from the town of Niort, close to the Sylve d'Argenson Natural Reserve (Réserve biologique Intégrale de la Sylve d'Argenson). This region is relatively dry, characterized by a brown soil on calcareous subsoil from old mesozoic sediment. The understory in the forest is dominated by *Rubus fruticosa*.

2.2 Biochar and phytometer plant species

The biochar was prepared from Scots pine (*Pinus sylvestris*) ca 90 %, birch (*Betula pubescens*) ca 5%, aspen (*Populus tremula*) ca 5 % and some traces of grey alder (*Alnus incana*), under 450 - 500°C charring temperature. It is a median temperature needed for charcoal formation (Glaser et al., 2002) and also the characteristic temperature for charcoal formation at ground level (Zackrisson et al., 1996) during common wild forest fires. I first sieved the biochar through a 2 mm and then through a 0.5 mm screen in order to standardize the size distribution (0.5 – 2 mm).

Two plant species (phytometers) were selected for their contrasting life-histories. (1) *Betula pendula* is a long-lived and relatively slow-growing perennial specie. It is suitable for the experiment because it is a pioneer specie which establishes quickly in the primary succession as well as in the secondary successional sequence after a fire disturbance (Ruokolainen and Salo, 2006). (2) *Phacelia tanacetifolia* is an annual herbaceous and relatively fast-growing specie, which originates from America but has a worldwide distribution thanks to its good adaptability to a wide range of climate and soil types. It was used for its low water demands, good germination, even at cool temperatures (3° - 20°C), and relatively fast flowering (6 – 8 weeks from germination) for comparatively long period (6 – 8 weeks) (Chen, 1966). I took the opportunity given by the relatively fast growth of *Phacelia*, reused the original soil and ran the experimental setup a second time (*see 2.4 Greenhouse experiment*).

2.3 Greenhouse experiment

The five soil samples from the different positions in each plot were pooled into one representative sample and homogenized. Each representative sample was divided into 4 pots (13 cm diameter). The first two pots were left unamended as control pots (i.e. no addition of biochar). The other two pots were used for biochar treatment by adding 2 g of size-standardized biochar particles (0.5 – 2 cm) to each pot. The biochar was mixed evenly into the soil in the pots. In total there were 60 pots in this experiment. The area of the pots was 133 cm^2 and the biochar addition corresponds to ca. 150 g of biochar per square meter, which is the average amount of biochar in the Scandinavian boreal forest ecosystem (Ohlson et al., 2009).

Each pot was marked with soil type, number of representative sample, and treatment. All pots were kept in a greenhouse under the following conditions: day night ratio 16 h:8 h; and day/night temperatures of 20/15°C respectively. Every second day approximately 15 l of water was added, divided equally over all pots. The pots were covered with plastic foil for two weeks in order to keep the soil constantly damp and so activate the biochar.

After the two weeks, ca. 20 *Betula* seeds were sown directly into each of the 15 pots with biochar treatment and into the 15 control pots (without biochar). The rest of the treatment and control pots were sown with 5-10 seeds of *Phacelia tanacetifolia* seeds. All the pots were kept covered with plastic foil and watered every second day with 15 l of water. The seeds began to germinate during the third week and the plastic foil covering was removed at the beginning of the fourth week (Photo 1 and 2). At this point the pots were watered by 15 l of water on a daily basis.

Photo 1 – No biochar control pots with *Phacelia* (left) and *Betula* (right) growing in arctic soil after the removal of the plastic covering (fourth week).

Photo 2 – Biochar treated pots with *Phacelia* (left) and *Betula* (right) growing in arctic soil after the removal of the plastic covering (fourth week)

The first harvest of *Phacelia* plants was collected after 66 days, when the first plants reached their flowering stage (Photo 5). Continuing the experiment would cause seed production, eventual mortification of various plant parts and subsequently the loss of biomass.

Since there was enough time to repeat the experiment with *Phacelia*, I decided to reuse the soil from the first experiment and add another 4 g of biochar, i.e. double the amount of biochar used in the first treatment, to test the effect of a higher biochar concentration in soil with lower nutrient content. In this second *Phacelia* experiment, the seeds were pre-sown into chemically inert pure sandy soil (Photo 3) and 5 seedlings were transplanted into the pots after they germinated and established seedlings. In addition 5 seeds were sown in each pot during the transplantation.

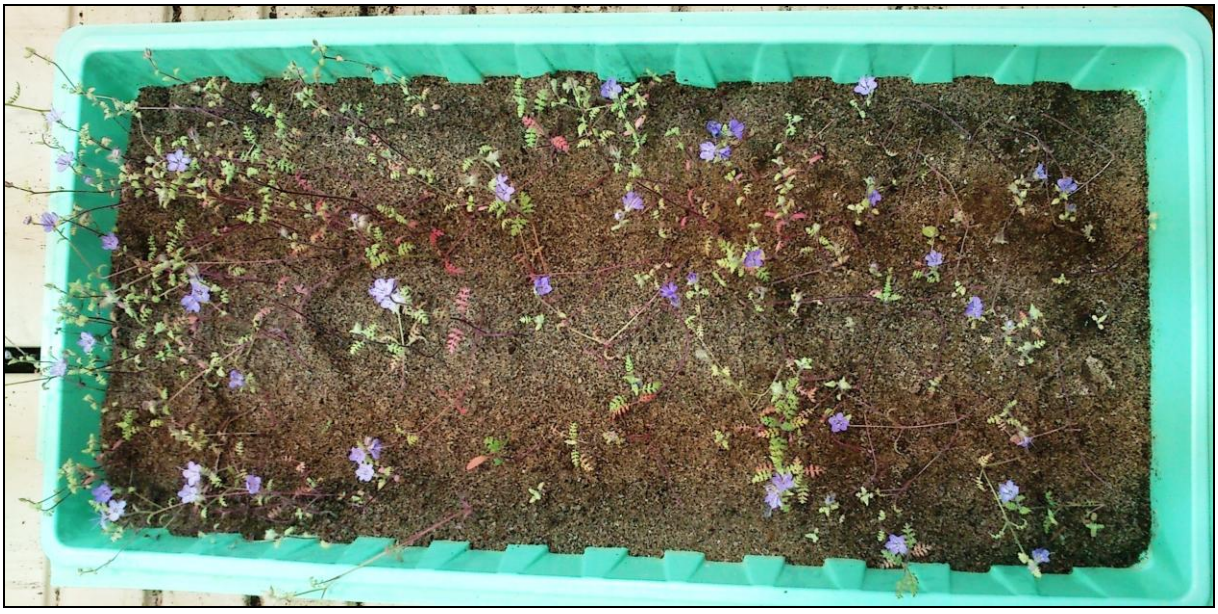


Photo 3 – Pre-sown *Phacelia tanacetifolia* plants

The second harvest of *Phacelia* plants was collected after 50 days when the first plants reached their flowering stage (Photo 6). *Betula* was harvested together with the second round of *Phacelia* (Photo 4). It was 116 days after the experiment began. At harvest, all plants were removed from their pots and the roots were cleaned of soil. The above and below ground parts were divided and stored separately in marked paper packets. All plant material was dried for 48 hours at 70°C and weighed.

Photo 4 – *Betula* before harvest

Photo 5 – *Phacelia* before the 1st harvest

Photo 6 – *Phacelia* before the 2nd harvest

2.4 Statistical analysis

This orthogonal experiment design is termed a 2 x 5 factorial for *Betula* and 2 x 4 for *Phacelia* with 6 replicate observations. In each of the 5 different geographical areas I picked six plots (replicates) and for each I used two types of treatment (biochar amendment and no biochar control). *Phacelia* is termed 2 x 4 because the specie was not capable to establish in the boreal forest soil at all. An ANOVA was computed separately for *Betula*, the 1st and 2nd harvest of *Phacelia*.

In order to assess the effect of each of the two crossed factors (biochar treatment and soil type) independently from each other and their interaction, a two-way ANOVA was run. Variables under consideration were: germination rate (number of emerged plants), above ground biomass, below ground biomass, total biomass, height, proportion of above ground to total biomass and proportion of below ground to total biomass.

Firstly, the pot specific average value for each variable was calculated to avoid pseudoreplication. Secondly, two outliers were removed from the dataset. One outlier was from the 2nd *Phacelia* harvest and the other one from *Betula* dataset (see Appendix 1). Both outliers were replaced by average values calculated from all remaining observations with the aim to retain equal replication in the experiment design. In order to follow the central limit theorem, variables were

converged to normal distribution by calculating square roots, where needed (See Appendix 2). Logarithm transformation could not be applied because of the occurrence of zero values in the datasets. Lastly, ANOVA was performed for every given variable under consideration. In conjunction with ANOVA Tukey simultaneous tests were used to compute all pairwise comparisons of the means of every treatment and find out which means were significantly different. Letters are used to indicate pairwise differences in the figures. A confidence interval of 95% (P=0.05) was used to perform all above mentioned tests. The entire collected dataset is presented as an electronic attachment (Appendix 1) as well as all statistical comparisons conducted by using MiniTab 16 (Appendix 2).

3. Results

The difference between the soil types was significant for all the used samples, with P values generally <0.001 (Tab. 1). There was no significant difference for number of emerged (germination rate) and height. The temperate soil had the highest overall productivity and also often demonstrated a significant soil type x treatment interaction effect (Fig. 1 and 2).

The main effect of treatment for total biomass was significant only for the two *Phacelia* harvests. In the 1st harvest the total biomass decreased significantly (P=0.044) while the 2nd harvest showed a significant increase (P=0.011). A general soil type x treatment interaction effect was significant only in the 2nd *Phacelia* harvest (P=0.045). *Betula* and the 2nd *Phacelia* harvest had significantly different interaction effect only in the temperate soil where the total biomass increased after biochar treatment.

The treatment effect for the above ground biomass was significantly different only for the 2nd *Phacelia* harvest (P=0.019) where the biochar amendment caused an increase. Even though interaction was generally non-significant for all the harvests, the temperate soil sample showed significant increase in the 2nd *Phacelia* harvest (Fig. 2).

The below ground biomass showed a significant impact of the treatment in all setups (Tab. 1). In the 2nd *Phacelia* (P=0.008) and *Betula* harvest (P=0.014) the main effect of treatment caused an increase of below ground biomass whereas in the 1st *Phacelia* harvest (P=0.022) the treatment had a significant decreasing effect. Even though, the general soil type x treatment interaction was non-significant for all harvests ($P \geq 0.09$), a significant difference of treatment in temperate soil sample for the 2nd *Phacelia* (Fig. 2) and *Betula* (Fig. 1) harvest can be observed.

The proportion of above ground to total biomass showed significant decrease by effect of treatment for *Betula* (P=0.005). An interaction effect was observed only for the 2nd harvest (P=0.024) with a decreasing trend in Mediterranean soil.

The main effect of treatment was observed in the proportion of below ground to total biomass causing significant increase for *Betula* (P=0.001) but decrease for the 1st *Phacelia* harvest (P=0.007). A significant increase in the Mediterranean soil sample of the 2nd *Phacelia* harvest was found, due to soil x treatment interaction.

Proportions above and below ground biomass to total biomass give the possibility to observe shoot to root ratios. In *Betula,* these decreased significantly in the biochar treatment, although the 1st *Phacelia* harvest indicates a significant increase. For the 2nd *Phacelia* harvest there was only a significant interaction effect in the Mediterranean soil where the shoot to root ratio decreased.

Betula

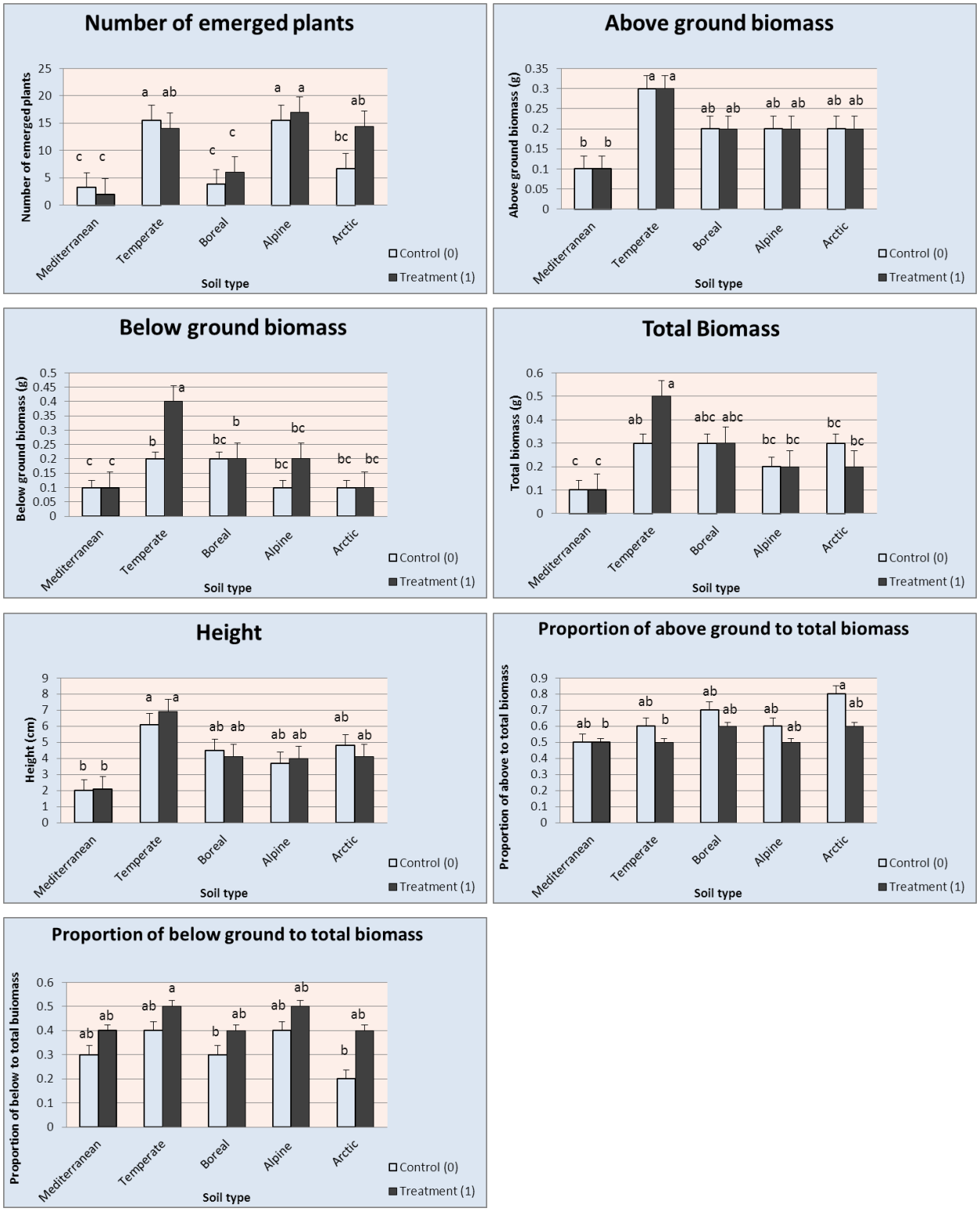
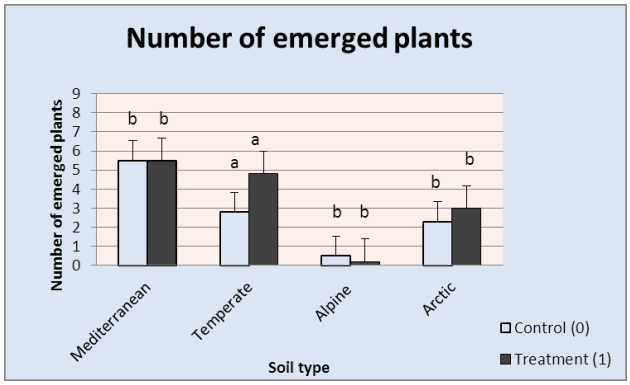
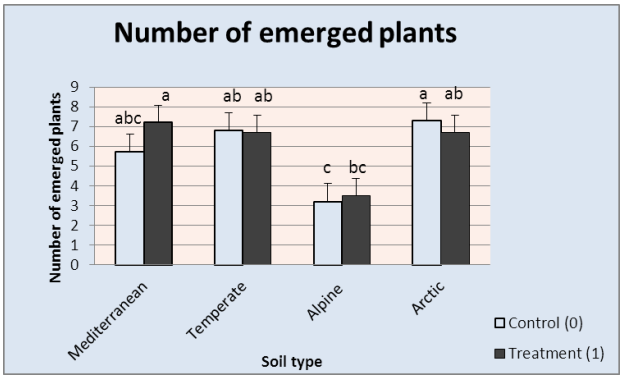
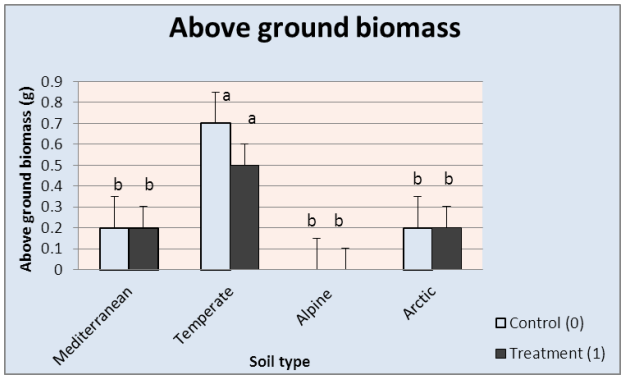


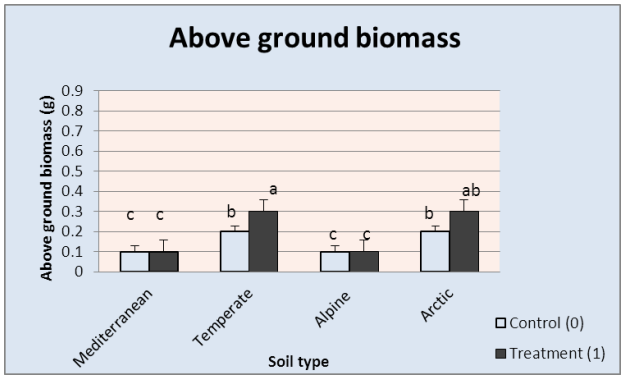
Figure 1 – Mean (+ 1 S.E.) of (1) germination rate (number of emerged plants), (2) above ground biomass, (3) below ground biomass, (4) total biomass, (5) height, (6) proportion of above ground biomass to total biomass, (7) proportion of below ground biomass to total biomass for *Betula pendula*. Means that do not share a letter within each graph are significantly different at P=0.05 (Tukey simultaneous tests following ANOVA on square root transformation where necessary - see Appendix 2).

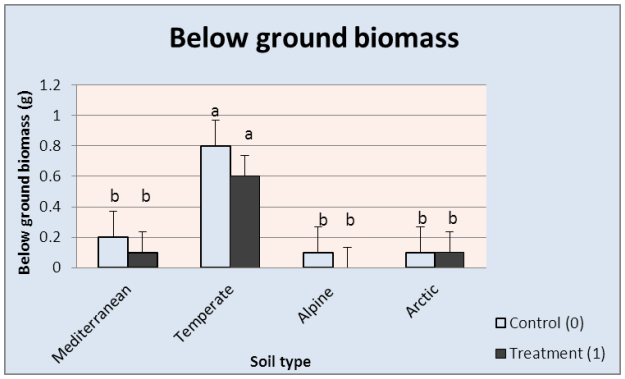
Phacelia 1st harvest	Phacelia 2nd harvest

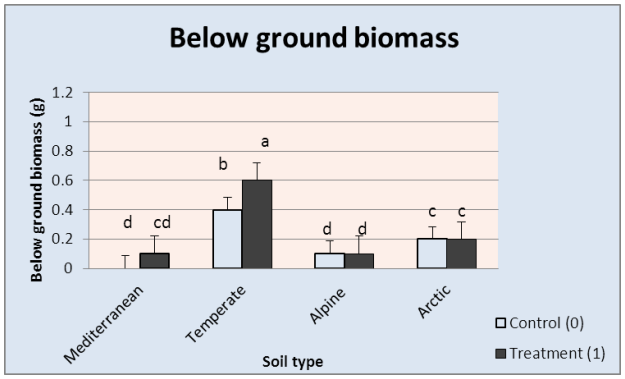


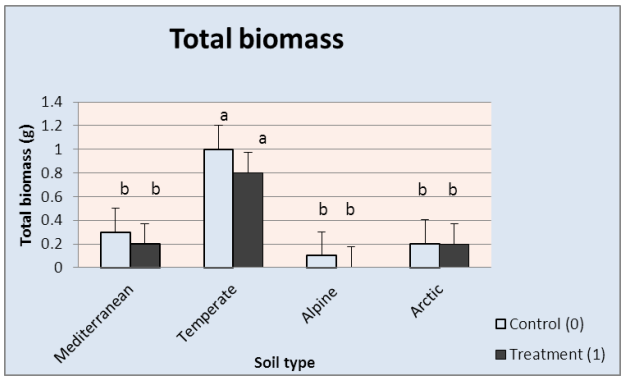


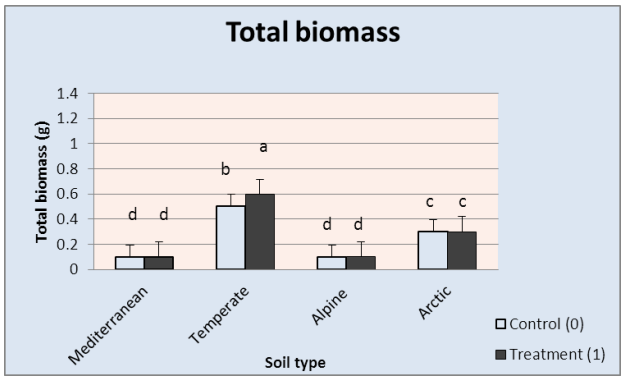












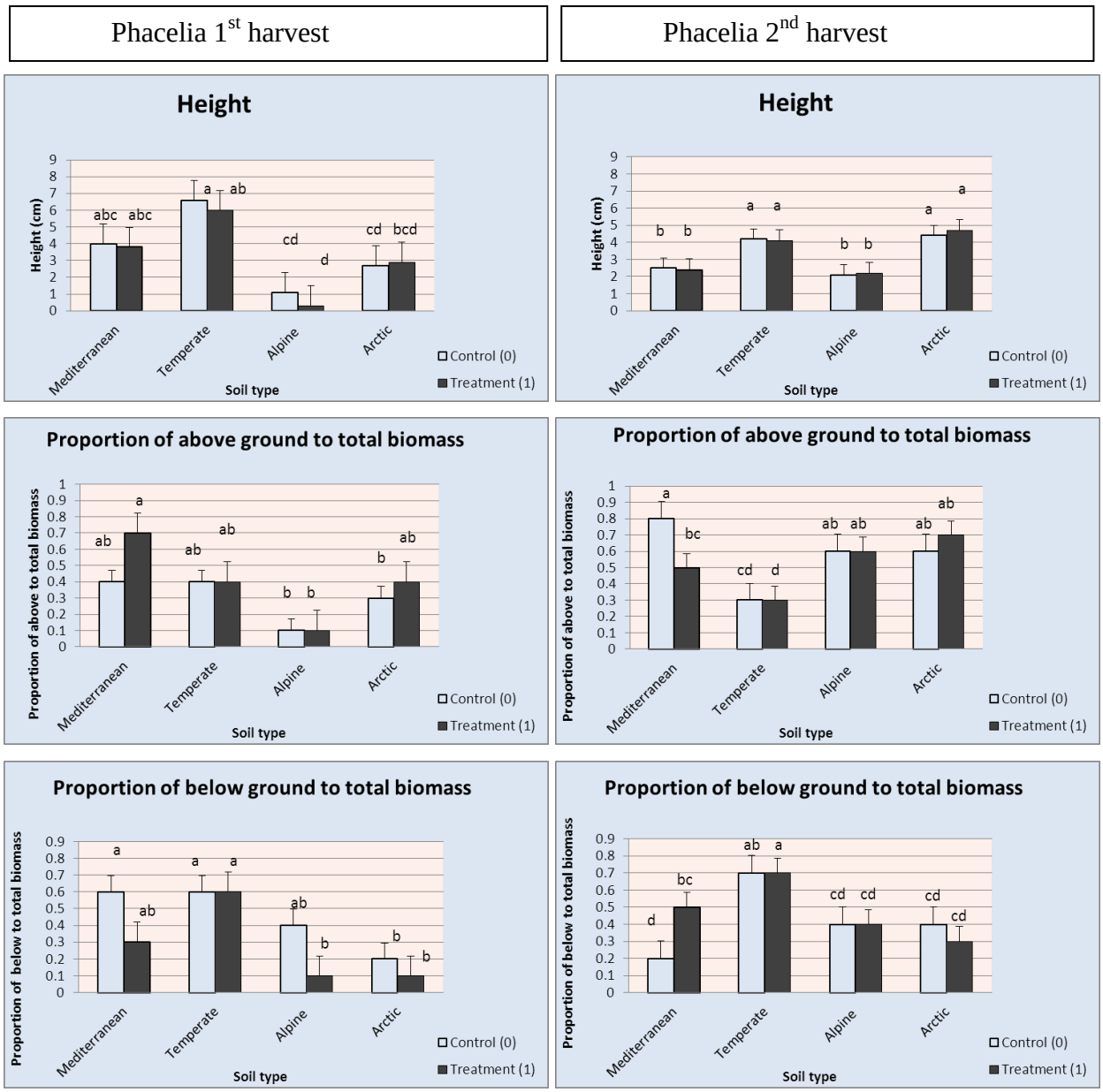


Figure 2 – Mean (+ 1 S.E.) of (1) germination rate (number of emerged plants), (2) above ground biomass, (3) below ground biomass, (4) total biomass, (5) height, (6) proportion of above ground biomass to total biomass, (7) proportion of below ground biomass to total biomass for 1st and 2nd *Phacelia tanacetifolia* harvest. Means that do not share a letter within each graph are significantly different at P=0.05 (Tukey simultaneous tests following ANOVA on square root transformation where necessary - see Appendix 2).

Table 1 – Analysis of variance result *Betula*, 1st and 2nd *Phacelia* harvest.

Terms for ANOVA (degrees of freedon in parenthesis) model are: *Betula* – soil type (4), treatment (1), soil type x treatment interaction (4); 1st and 2nd *Phacelia* – soil type (3), treatment (1), soil type x treatment interaction (3);

	Phacelia 1st harvest		**Phacelia 2nd harvest**		**Betula**	
RESPONSE VARIABLE	**F**	**P**	**F**	**P**	**F**	**P**
No.of emerged						
Soil	12.27	<0.001	10.51	<0.001	25.61	<0.001
Treatment	0.89	0.352	0.24	0.627	2.74	0.104
Interaction	0.69	0.562	0.77	0.519	2.47	0.056
Above ground						
Soil	50.74	<0.001	130.43	<0.001	9.05	<0.001
Treatment	3.06	0.088	6	0.019	0	0.948
Interaction	1.67	0.188	2.27	0.095	0.51	0.726
Below ground						
Soil	83.85	<0.001	90.47	<0.001	18.87	<0.001
Treatment	7.86	0.008	5.67	0.022	6.42	0.014
Interaction	0.9	0.451	2.3	0.092	2.13	0.09
Total biomass						
Soil	66.55	<0.001	121.75	<0.001	12.88	<0.001
Treatment	4.33	0.044	7.17	0.011	0.98	0.327
Interaction	0.9	0.451	2.94	0.045	1.16	0.339
Height						
Soil	22.29	<0.001	184.71	<0.001	9.08	<0.001
Treatment	0.62	0.434	0.56	0.46	0	0.984
Interaction	0.19	0.905	0.88	0.46	0.31	0.87
Prop. of above*						
Soil	9.76	<0.001	19.34	<0.001	3.46	0.014
Treatment	1.6	0.214	2.59	0.116	8.49	0.005
Interaction	1.65	0.192	3.5	0.024	0.35	0.849
Prop. of below*						
Soil	11.61	<0.001	19.34	<0.001	2.98	0.028
Treatment	8.2	0.007	2.59	0.116	12.54	0.001
Interaction	1.29	0.291	3.5	0.024	0.52	0.721

*Two zero values, one in *Betula* and the other one in the 1st *Phacelia* harvest dataset, caused an inconsistency in the results of the proportion of above ground biomass to total biomass and proportion of below ground biomass to total biomass. In both of these variables the values of P and F should be equal, as it is in the 2nd *Phacelia* harvest.

4. Discussion and conclusion

I had expected that biochar addition would have a generally positive effect on plant productivity, with highest impact in the least productive soil types. However, this was not the case. This study does not confirm any significant difference between fertile- and less fertile soils in terms of their response to biochar addition. In contrast to the insignificant effect of biochar addition, the effect of soil type was very significant and the results confirm the hypothesis that there was a clear difference between the soil types with regard to fertility and plant productivity. The temperate soil had the highest productivity with, as well as without biochar, which indicates that the temperate soil type had a higher nutrient content and better structure than the other soil types. The temperate soil was also the only substrate which showed a highly significant level of soil type x treatment interaction, with a variable biochar effect depending on study specie, harvest and measured variable. Against all expectations, no significant results were observed for plant height and germination rate (number of emerged plants), although for example Vookova and Kormutak (2001) reported up to a 70% increase in germination (radicle elongation) of *Abies numidica* somatic embryos, following the addition of activated biochar to different germination mediums.

The only relatively consistent and significant result for biomass is found in the 2nd *Phacelia* harvest. Here, the below ground, above ground and hence also total biomass showed significantly increasing effect after the biochar treatment. However, there was no significant effect on shoot to root ratio. This is in agreement with multiple studies which indicate that biochar can enhance net primary production (e.g. Rajkovich, 2012; Laird, 2008; Lehmann et al., 2006; Lehmann, 2007a; Zackrisson et al., 1996). It has also been suggested that biochar may bring potential risks (Lehmann et al., 2011). This statement is supported by the results from the 1st *Phacelia* harvest, where the below- and total biomass was significantly lower after the biochar treatment as compared with the control. Lehmann et al. (2011) suggested that an increase of the shoot to root ratio, as can be observed for the 1st *Phacelia* harvest, combined with the aforementioned biomass decrease, indicates a direct toxic effect of biochar on the root system. Yet, no such negative results of biochar additions have been previously published (see Lehmann et al., 2011).

Betula showed a significant increase only for the below ground biomass following biochar addition. The fact that there is a significant difference in this variable also for *Phacelia* indicates a

clear impact of biochar on the root system dynamics, the mechanisms of which are not yet well understood (Lehamnn et al., 2011). The increase in the below ground biomass after biochar augmentation, which was observed for *Betula* and the 2nd *Phacelia* harvest, is in agreement with several existing reports (e.g. Breazeale, 1906; Wardle et al., 1998; Rodríguez et al., 2009; Yin et al., 2012). Lehamnn et al. (2011) attributed this effect to biochar's ability to improve soil properties, such as porosity and pH, which may indirectly improve root growth. However, other researchers documented a decrease of root biomass (e. g. Brockhoff et al., 2010; Prendergast-Miller and Sohi, 2010), which corresponds with a theoretical increase in nutrient availability to plants after biochar addition. In my study, the simultaneous decrease of the shoot to root ratio (significant for *Betula* and insignificant for the 2nd *Phacelia* harvest) suggests that this phenomenon is more likely an effect of lower resource supplies and biochar properties (Lehmann et al., 2011). This correlation between nutrient deficiency and root system expansion was described by Wilson (1987), who explained that the plant needs to invest relatively more resources into the root system in order to reach for water and nutrients. In this study, the results may be caused by competition for resources between the *Betula* plants, which were growing in one pot, and a possible depletion of nutrients after the cultivation of the 1st *Phacelia.*

By contrast, several studies report a higher shoot to root ratio combined with increased biomass and greater nutrient uptake efficiency in the presence of biochar (e.g. Wardle et al., 1998; Pettersson et al., 1993). This was shown for example by Wardle et al. (1998) in an experiment where *Betula pendula* had more than six times higher uptake of nitrogen, significantly higher total biomass and enhanced shoot to root ratio after biochar augmentation on a site with ericaceous vegetation and acidic, nutrient poor soil. The authors interpreted this effect as a result of sorption of allelochemicals (phytotoxic compounds) produced by ericaceous species which can have a deleterious impact on plant growth (Zackrisson et al., 1997). However, no such result was noted in my study. For example, the boreal soil, which is ultimately an ericaceous substrate, showed no significant increase of *Betula* biomass after biochar addition.

The weak and erratic impact of biochar in my study may be caused by various factors. These may influence the effect of biochar on soil properties and yield (Lehmann et al., 2011; Keech et al., 2005; Glaser et al., 2002). It was suggested that pH, electric conductivity, sorption, structure, physical properties and the overall chemical composition of biochar (Lehmann et al., 2011; Gundale and DeLuca, 2006) may be influenced by charring temperature (Elad et al., 2011; Gundale and DeLuca, 2006; Keech et al., 2005), source materials (Gundale and DeLuca, 2006; Rajkovich, 2012), size of biochar fragments (Scott and Damblon, 2010) amount of biochar

(Glaser et al., 2001) and fertilizer addition (Steiner et al., 2007; Ogawa et al., 2006). Other influencing factors may be soil type (Steinbeiss et al., 2009; Glaser et al., 2002), duration of biochar treatment (Lehmann et al., 2011) and the choice of plant species (phytometers).

In order to try to identify some factors that are likely responsible for the divergences in the results, we can juxtapose the current study with that of Wardle et al. (1998) and note following differences: (i) twigs of *Empetrum hermaphroditum* used as biochar feedstock; (ii) addition of half the amount of biochar (1g) while the pots were smaller (8 cm diameter); and (iii) a longer duration of the experiment (harvested 57 days after planting or 133 days after biochar augmentation). However, there were also several common factors: (i) charring temperature (450°C), (ii) use of the same perennial phytometer (*Betula*), (iii) no fertilizer addition and (iv) a similar size distribution of the biochar particles (0.5 – 1.6 mm).

Differences between my study and that of Wardle et al. (1998) seem to be a plausible explanation for the varying results between the harvests in this study. The only variables in which *Betula*, the 1st *Phacelia* and the 2nd *Phacelia* harvests diverge between each other are: the amount of biochar (4g were added for the 2nd *Phacelia* harvest), the time span between first biochar addition and sowing (1st *Phacelia* & *Betula*: 14 days, 2nd *Phacelia*: 80 days) and amount of nutrients during planting (2nd *Phacelia* must have had less nutrients because they were used by the 1st harvest, *Betula* plants could have been competing for resources within one pot). For example, the effect of the amount of biochar on results was described by Glaser et al. (2001), who found that low amounts of biochar stimulated the yield positively, whereas higher amounts had a negative effect on the yield. Nonetheless, this result is inconsistent with the present study where the 1st *Phacelia* harvest was the only one demonstrating signs of toxicity although it had the same or lower biochar dose as compared to *Betula* and the 2nd *Phacelia* harvest, respectively.

The results of my study are very hard to explain but they seem to be a function of biochar properties, nutrient content and time. Possibly, the used biochar was somewhat phytotoxic in contact with some compound in the soil (e.g. a nutrient) but this effect decreased with time as the amount of nutrients decreased. This would also explain why the toxicity effects were not observed even after the addition of another biochar dose, since the level of nutrients was already lower. Should this be the case and the toxicity effect disappeared, it would also explain the ob-

served positive effect of biochar, as an enlargement of the root system, due to lasting low nutrient supplies.

In light of the results of my study, and following the CSIRO report (Sohi et al., 2009), I would like to join Lehmann and colleagues, who call for systematic research to develop the characterization of a minimum set of specific biochar properties, such as:

> "*microbially available C, surface area, pore size distribution, pH, ash content, and elemental analyses as well as production conditions (temperature and time at highest temperature) and feedstock type. In addition, contrasting biochars have to be compared rather than one biochar studied on its own. Knowledge gaps needing urgent attention include biochar effects on faunal abundance (especially micro- and meso-fauna), on the ecology of biota including environmental risk, on electrochemical properties as well as on the utility as inoculant carriers, on interactions with enzymes and for managing plant pathogens. On the short term, characterizations standards need to be developed that adequately capture the most important differences in biochar properties starting with those mentioned above*" (Lehmann et al., 2011).

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