Norwegian University of Life Sciences
Faculty of Biosciences
Department of Plant Sciences

Philosophiae Doctor (PhD)
Thesis 2021:64

Yield and quality of vegetables fertilized with materials recycled from organic resources

Avling og kvalitet hjå grønsaker gjødsla med materiale resirkulert frå organiske ressursar

Ingunn Øvsthus

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Ås/Ullensvang 2021

Thesis number 2021:64
ISSN 1894-6404
ISBN 978-82-575-7838-7

Year	2021
Title	Yield and quality of vegetables fertilized with materials recycled from organic resources
Author	Ingunn Øvsthus
Publisher	Norwegian University of Life Sciences Faculty of Biosciences Department of Plant Sciences
Funding	This PhD Thesis was a part of the project *Pre- and postharvest quality optimisation of organic vegetables that can stimulate an increased consumption* (NFR 176767), which was led by researcher Dr. Randi Seljåsen, Norwegian Institute of Bioeconomy Research (NIBIO) and funded by the Research Council of Norway, The Council of Nordland and Troms, and NIBIO.
Thesis number	2021:64
ISSN	1894-6402
ISBN	978-82-575-1838-7

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ACKNOWLEDGEMENT

This thesis is written for the Degree Philosophiae Doctor at the Norwegian University of Life Sciences. The work has been carried out during my period as PhD student associated to the project – *Pre- and postharvest quality optimisation of organic vegetables that can stimulate an increased consumption* (NFR 176767) at Norwegian Institute of Bioeconomy Research, Division Bodø during the years 2007 to 2012. The thesis is financial supported by Norwegian Research Council (NRC), the counties of Nordland and Troms, the Norwegian institute of Bioeconomy Research. Finishing this thesis has been a long path for me; balancing health challenges with progress, and in recent years the work has been completed without funding during weekends and holidays.

First of all, I thank my main supervisor prof. Dr. Tor Arvid Breland for supervision throughout the PhD period, and for his patience and valuable advice. His careful and accurate way of working has inspired me and helped me to develop myself toward this scientific degree. Thanks to co-supervisor Dr. Anne-Berit Wold for giving me valuable feedback on my thesis.

I thank also co-supervisor and project leader Dr. Randi Seljåsen for always having time to discuss and give me feedback, as well as the project participants Dr. Elizabeth Stockdale, Dr. Kirsten Brandt and Karl Christensen at The University of Newcastle for their hospitality during my study periods in England. Thanks also to Dr. Gunnar Bengtson for being my contact person at NOFIMA, where the glucosinolates analysis was conducted, and to Dr. Sidsel Fiskaa Hagen for guiding me through the steps of NOFIMA's internal protocol of glucosinolate analysis. Dr. Trond Knapp Haraldsen is thanked for providing meat bone meal, fish waste sludge, anaerobically digested food wastes, as is Dr. Tor J. Johansen for providing shrimp shell powder and shrimp shell pellets. Kristian Thorup-Kristensen is thanked for his good advices and supervision associated with the use and calibration of the EU-Rotate_N model. Peter Dörsch is thanked for always being positive and motivating, and for organizing and supervising the carbon dioxide and nitrous oxide measurements at the NMBU laboratory. I will also thank all co-authors for giving me valuable feedback on the manuscripts. A special thanks to Dr. Hugh Riley for improving the language of the thesis.

Without technical support and assistant from Mr. Per Magnus Hansen to conduct the field experiment, this work would have been impossible. I also wish to thank colleagues in NIBIO for their friendliness.

Last, but not least, I wish to thank my friends and family for their support and encouragement throughout this period. A special thanks to my children Synne and Andreas for the giving me the time to complete my thesis.

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SUMMARY

Agriculture, aquaculture, fishery and households generate large amounts of organic wastes with high contents of nitrogen (N) and other nutrients. Concurrently, supply of off-farm N resources into horticultural production systems is essential to gain desirable yields, quality and economic outcome. Turning organic wastes into fertilizer resources can contribute to meeting the requirement of nutrients without consuming non-renewable resources will contribute to "closing the loop" and thus a more circular economy recycling nutrients from such locally available organic resources.

However, recycling nutrients from organic materials is a complex task, and knowledge about nutrient dynamics is important for optimizing fertilizer effect without causing detrimental impacts on the environment. In particular, the N dynamics of organic materials requires substantial attention, due to the complexity of pathways in the N cycle and their potentially negative impacts on the environment. These processes depend upon the biochemical quality of the organic fertilizer materials and external factors such as temperature and moisture and soil texture and structure. There is a risk of loss of N through nitrate leaching, ammonia volatilization or fixation, and denitrification.

Horticultural products are an important nutritional source for humans. Vegetables, fruit and berries are associated with a healthy diet. Fertilization strategy influences both internal and external product quality, and especially N fertilization is linked to yield and, hence, economic profit, as well as contents of nutritional value and taste. Knowledge about the N mineralization and immobilization from organic fertilizer resources is required to ensure a high degree of resource utilization and optimal quality of the horticultural produce. N models have been widely used to increase our understanding of how N dynamics influences the yield and environmental impact in both conventional and organic production systems.

The overall aim of this thesis was to investigate the effect of fertilization with materials recycled from organic resources on yield and quality of selected vegetables. An incubation experiment with nine organic materials of different origin (anaerobically digested food wastes (AD), shrimp shell pellets (SSP), shrimp shell powder (SSM), meat bone meal (MBM), dried fish waste sludge (FW), sheep manure (SM), algal meal (AM) and meals of *Laminaria digitata* (LD) and *Saccharina latissimi (SL)*) was set up to determine the carbon (C) and N mineralization patterns. Broccoli, potato and lettuce were grown at two locations, Grimstad (58°N and 8°E) and Bodø

(67°N and 14°E), with anaerobically digested food wastes, shrimp shell pellets, sheep manure and algal meal as fertilizers to investigate effects on yield, N use efficiency and selected quality parameters. The C and N mineralization data obtained during incubation and results from the field experiment in Bodø were used to calibrate and evaluate the EU-Rotate_N model. Based on net N mineralization, the organic materials were divided into three groups: N-rich industrial wastes which had a high initial N mineralization rate followed by a low rate (SSP, SSM, FW, MBM), materials with high initial mineral N content and further low rate of N mineralization (AD and SM), and seaweeds, which caused initial N immobilization followed by slow (SL and LD) or no (AM) N mineralization. Crop yield, N recovery efficiency and crop quality parameters could to a large extent be explained by the plant-available N from the different fertilizer materials as estimated from the mineralization data. However, sensory attributes of broccoli were affected by years. EU-Rotate_N was successfully calibrated for N-rich materials of industrial origin, whereas seaweeds, AD and SM proved to be difficult. The model's ability to predict was evaluated with soil and crop data of broccoli and potato fertilized with AD, SSP, SM, AM, and mineral fertilizer (MF). The model satisfactorily predicted dry matter and N contents of the above-ground part of broccoli fertilized with AD, SSP and MF, but not AM, and of potato after adjusting critical %N for optimum growth. Prediction of soil inorganic N after harvest was poorer.

In conclusion, the N-rich organic materials of industrial origin (SSP, SSM, MBM and FW) and AD have the potential to replace N from mineral fertilizer in conventional vegetable production systems or as complementary fertilizers in organic production systems. The decomposition of and N availability from seaweed species were not fully understood. The EU-Rotate_N model can be used as a learning tool for understanding the decomposition and N mineralization dynamics of organic materials and, thus, serve as a decision support tool for their use as fertilizers.

SAMANDRAG

Landbruk-, fiskeri- og havbruksnæringar, og hushald produserer store mengder organisk avfall med høgt innhald av nitrogen (N) og andre næringsstoff. Samtidig er det trong for tilført N til produksjonssystema i landbruket for å oppnå ynskt avling, kvalitet og økonomisk profitt. Utnytting av organisk avfall som gjødselressurs kan bidra til å dekke trongen for næringsstoff i både økologiske og konvensjonelle produksjonssystem. På denne måten reduserer ein forbruket av ikkje-fornybare ressursar og ein unngår tap av verdifulle næringsstoff. Sirkulær økonomi og resirkulering av næringsstoff frå lokalt tilgjengelege organiske avfallsressursar står høgt på den politiske agendaen.

Resirkulering av næringsstoff frå organiske avfallsressursar er utfordrande. Kunnskap om mineraliseringsmønster er derfor nødvendig for å oppnå optimal gjødseleffekt og minimal negativ innverknad på miljøet. Det har særleg vore retta fokus mot kompleksiteten i N-dynamikken ved nedbryting av organisk materiale. Omgjering av N i organisk form til plante-tilgjengeleg form er avhengig av dei biokjemiske eigenskapane til det organiske materialet. Prosessane er og avhengig av ytre faktorar som temperatur og råme, samt jordtekstur og -struktur. Det er stor risiko for å miste N gjennom prosessar som utvasking av nitrat, denitrifisering, tap av ammoniakkgass og N-fiksering dersom ikkje tidspunktet for frigjeving av N stemmer med plantene sitt utviklingstadium med trong for næringsstoffet.

Frukt, bær og grønsaker har viktig ernæringsmessig verdi for menneske. Mange relaterer konsum av hagebruksprodukt med eit sunt kosthald. For å oppnå rett kvalitet og næringsverdi er det viktig med kunnskap om korleis ulike gjødslingsstrategiar verkar inn på produktet, men også for å sikre berekraftig forvalting og høg utnyttingsgrad av gjødselressursane. N-modellar er mykje nytta verktøy for å forstå N-dynamikken og korleis bruken verkar inn på avling og miljø i ulike produksjonssystem.

Det overordna målet med denne avhandlinga har vore å undersøke gjødslingseffekten av organiske gjødselressursar, og korleis bruken påverkar avling og kvalitet på utvalde grønsaker. Eit inkubasjonsforsøk med ni organiske gjødselressursar av ulikt opphav (rest frå biogass produksjon basert på matavfall (AD)), pellets av rekeskal (SSP), rekeskalmjøl (SSM), kjøttbeinmjøl (MBM), tørka fiskesslam (FW), sauegjødsel (SM), algemjøl (AM) og mjøl av *Laminaria digitata* (LD) og *Saccharina latissimi* (SL)) vart gjennomført for å bestemme

karbon- (C) og N-frigjevingsmønster. Feltforsøk med brokkoli, potet og salat vart gjennomført i Bodø og Grimstad for å undersøke effektar på avling, plantene si N-utnyttingsgrad og utvalde kvalitetseigenskapar etter gjødsling med AD, SSP, SM, og AM. C- og N-mineraliseringsdata frå inkubasjonsforsøket og resultat frå feltforsøket vart nytta til å kalibrere og evaluere N modellen EU-Rotate_N. Basert på netto N-mineralisering vart dei testa organiske gjødselressursane delt inn i tre grupper: industrielt avfall med høgt-N innhald og høg N mineraliseringsrate i starten etterfylgt av låg rate (SSP, SSM, FW, MBM), høg grad av mineralsk N ved oppstart av forsøket og vidare låg mineraliserinsrate (AD og SM), og tang og tare, som hadde immobilisering av N i starten etterfylgt av langsam frigjeving (SL og LD) eller ingen (AM) N-mineralisering. Avlingsutbytte, N-utnyttingsgrad og produkta sine kvalitetseigenskapar kan i stor grad forklarast med estimert plant-tilgjengelege N frå gjødselmateriala. Sensoriske eigenskapar for brokkoli var derimot meir påverka av år. Kalibrering av EU-Rotate_N modellen var vellukka for dei N-rike organiske materiala av industrielt opphav, medan for tang og tare, AD og SM var kalibreringa utfordrande. Modellen sin evne til å føreseie avlingsdata for brokkoli og potet gjødsla med AD, SSP, SM, AM og mineralgjødsel (MF) vart evaluert. Modellen predikerte tilfredsstillande tørrstoffavling og N-innhald for brokkoli gjødsla med AD, SSP og MF, men ikkje AM. Predikering av potetavling og N-innhald var bra etter justering av modellen si kritisk% N for optimal vekst, medan predikering av mineralsk N i jord etter hausting var dårleg.

Ein kan konkludere med at dei N-rike organiske materiala av industrielt opphav og AD har potensialet til å erstatte N frå mineralgjødsel i konvensjonelle grønsaksproduksjon eller som tilleggsgjødsel i økologiske produksjonssystem. Vi treng meir kunnskap om nedbryting og N-frigjeving frå tang- og tareartar. EU-Rotate_N modellen kan nyttast som verktøy for å lære om N-dynamikk ved nedbryting av organisk materiale. Modellen kan og nyttast av dyrkingsrådgjevarar og forvaltarar som skal ta viktige avgjersler.

LIST OF PAPERS

Paper I

Øvsthus I, Breland TA, Hagen SF, Brandt K, Wold AB, Bengtsson GB and Seljåsen R, 2015. Effects of organic and waste-derived fertilizers on yield, nitrogen and glucosinolate contents, and sensory quality of broccoli (*Brassica oleracea* L. var. *italica*). Journal of Agricultural and Food Chemistry 63:10757−10767

Paper II

Øvsthus I, Seljåsen R, Stockdale E, Uhlig C, Torp T, Breland TA, 2017. Yield, nitrogen recovery efficiency and quality of vegetables grown with organic waste-derived fertilisers. Nutrient Cycling in Agroecosystems 109(3):233–248

Paper III

Øvsthus I, Thorup-Kristensen K, Seljåsen R., Riley H, Dörsch P and Breland TA, 2021. Calibration of the EU-Rotate_N model with measured C and N mineralization from potential fertilizers and evaluation of its prediction of crop and soil data from a vegetable field trial. European Journal of Agronomy, in review; revised and resubmitted.

Paper IV

Johansen TJ, Samuelsen TA and Øvsthus I, 2019. Growth and nitrogen recovery efficiency of potato (*Solanum tuberosum*) fertilised with shrimp shell pellets. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 69(7):559–566

ABBREVIATIONS

SSP	Shrimp shell pellets
SSM	Shrimp shell powder
AM	Algal meal
LD	Algal meal *Laminaria digitata*
SL	Algal meal *Saccharina latissima*
FW	Fish sludge waste
MBM	Meat bone meal
AD	Anaerobically digested food waste
SM	Sheep manure
NRE	Nitrogen recovery efficiency
AOM	Added organic material
AOM_slow	Fraction of slowly degradable added organic material
AOM_fast	Fraction of easily degradable added organic material
SMB	Soil microbial biomass
SOM	Soil organic matter
*k*_slow	Decomposition rate coefficient of slowly degradable fraction
*k*_fast	Decomposition rate coefficient of easily degradable fraction

1. INTRODUCTION

1.1 The challenge of sustainable fertilizer use in vegetable production

Agricultural and horticultural crops production depends upon the use of mineral fertilizers to meet crop nutrient requirements. In 2017, the consumption of nitrogen (N) from mineral fertilizers in Norway and Europe corresponded to 103,800 and 11,300,000 Mg, respectively (Eurostat 2017). Of which consumption for vegetable and root & tuber production correspond to 4% of European N fertilizer use in 2014 (Heffer et al 2017). The economic outcome per unit area is high for this sector of agriculture. To ensure high yield of this valuable production, mineral fertilizers are often supplied in excess of crop requirements (Tei et al 2020). This contributes to a relative low N use efficiency for vegetables and a high risk of losing N to the environment.

Concurrently to the intensive use of mineral fertilizer, agriculture, aquaculture, fishery and households generate large amounts of organic wastes containing N and other valuable nutrients. Potentially, these waste resources can be utilized as fertilizers in horticulture. Use of organic wastes as a supplement to mineral fertilizer in conventional production systems may contribute to reducing the accumulation of reactive N in the environment (Galloway 2003; 2008), reducing energy demand (e.g., for N fixation by the Haber-Bosch reaction and for transportation) and reducing the demand for non-renewable resources (e.g., phosphorous (P) (Brod et al 2015a; 2015b)). When managed properly, they may promote soil fertility and increase microbial activity in the soil ecosystem (Diacono and Montemurro 2010). The organic materials can also be utilized in organic farming systems. In such production systems, plant nutrient requirements should ideally be covered by the design and management of locally adapted agroecosystems (IFOAM 2014), preferably by use of farm-internal N_2 fixation, animal manure and green manure. Additional off-farm-resources may be needed, especially on stockless farms and when producing horticultural products with high N demand (e.g., *Brassica spp.*; Möller 2018).

Proper use management of organic materials as N fertilizer resource for conventional and organic vegetable production requires knowledge about the fertilizer potential. Potentially, N mineralization from organic materials can be determined by biological and chemical methods. Incubation experiments under standard environmental conditions (Sharifi et al 2007; Jensen et al 2005) or in the field (Lehrsch et al 2016) and recording N uptake in crops fertilized with the organic materials (Constantin et al 2011) are examples of biological methods to estimate the N fertilizer value of organic materials. The transfer value of the N mineralization patterns obtained under *in situ* methods are restricted as the reality is more complex. Knowledge-transfer obtained in laboratory small-scale N mineralization studies into "real-conditions" can be done by use of models, which account for climate conditions and soil properties. The up-scaling of knowledge into site-specific, may need model parameterization (Manzoni and Porporato 2009; Cambell and Paustian 2015).

1.2 Organic materials with potential for recycling as fertilizer

In Norwegian fisheries and aquaculture industries, the amount of residual raw materials is increasing. In 2016, residual material was estimated at 909,742 Mg, including wastes from whitefish (cod and herring) offshore fishing, pelagic fish, aquaculture, and shellfish (shrimps and crabs) (Richardsen 2017). In 2016, 100% and 91% of the residual raw materials from pelagic fish and aquaculture, respectively, as utilized as feed ingredients and as human food (oil, cod liver oil, seafood products and extracts). The whitefish and shellfish industries have a lower utilization rate: 44% and 28%, respectively. In the whitefish industry, fish processing wastes is done onboard the fishing boat and not on land due to the lack of technology to take care of wastes. Also, in the mussels, crab and shrimp industries, the utilization of wastes could be further developed (Richarden et al 2017). These aquaculture and fishery waste materials are generally rich in nutrients, especially N and phosphorus (P).

In addition to the above-mentioned wastes from the fishery and aquaculture industries, these industries contribute to a great nutrient flow from feed and faeces (fish sludge) into the environment around aquaculture cages. The effluent contains organic and inorganic substances with carbon (C), N and P (Wang et al 2012). There are considerable amounts of unrecorded waste related to excess feed and faeces. 62–70% of the total N and P in feed inputs are unutilized and remain in the water (Wang et al 2012). Concurrently, the aquaculture industry is growing, and it is estimated that the Norwegian aquaculture industry will increase fivefold (Olafsen et al 2012). Then the amount of organic waste and nutrients from fishery and aquaculture will increase substantially. A considerable amount of fish sludge would then potentially be available for fertilizer purposes. Considering its high contents of N and P (7–8% and 2–3%, respectively), the fish sludge is a valuable fertiliser resource in agriculture. The fertilizer effect of fish sludge has previously been studied (e.g., Brod et al 2012; 2014; 2017). Dried and digested fish sludge supplied to barley resulted in a relative agronomic efficiency of supplied N (unit of yield response per unit of N applied) of 50-80% compared to mineral fertilizers (Brod et al 2017). Today, Norwegian pollution regulations include restrictions for wastes and discharges to sea from on-land hatcheries and fish processing (*Forurensningsloven* and *Forurensningsforskriften*; Norwegian Ministry of Climate and Environment 2004). However, surplus fish feed and faeces in open marine systems are difficult to collect and national regulations do not currently exist.

For open aquaculture systems, macroalgae, e.g., seaweed, may be used as a biofilter to capture inorganic N and dissolved nutrients in seawater (bioremediation and integrated multi-trophic aquaculture, Reid et al 2013; Fossberg et al 2018). This integrated cultivation method has been suggested to prevent nutrients from entering the environment. In addition, by-products from macroalgae, e.g., energy production by biogas digestion, bioethanol fermentation, fertilizer, soil conditioners, animal feed and various human cosmetics, food, and medical products (Roesijadi et al 2010) may all be potentially profitable. Macroalgae are utilized in horticultural production as fertilizer, as soil conditioners or biostimulants in fresh, dried, composted forms or as extracted compounds (reviewed by Battacharyya et al 2015), and have been shown to have positive effects on growth and stress tolerance of plants and to improve soil texture and water-holding capacity (Blunden 1991; Spann and Little 2011; Khan et al 2009; Alobwede et al 2019; Haslam and Hopkins 1996). The N contents in macroalgae vary from 1 to 3% and the C:N ratio ranges from 17 to 33 depending on species (Øverland et al 2018). Thus, utilization of such materials for agricultural purposes requires knowledge about fertilizer effect and nutrient recycling in order to ensure timing of mineralization according to plant requirement.

Agriculture also contributes to a considerable amount of organic waste materials which has a potential as fertilizer, e.g., slaughterhouse wastes, plant residues from vegetable or arable crops, and animal manure. Traditionally, crop residues and animal manure have been utilized as nutrient resources and are still a valuable but often under-utilized nutrient source in agriculture partly due to a regionalization of animal and crop productions, respectively. Field and laboratory experiments have been conducted to increase knowledge about management practice for optimal fertilizer utilization. Meat-bone meal (MBM), which is dried slaughterhouse wastes, have been used as protein and mineral nutrition sources for livestock. After the occurrence of transmissive spongiform encephalopathies (TSE), which was associated with MBM feeding of ruminants, the traditional utilization of this by-product was banned (European commission, 2000). Use of MBM as fertilizer was permitted by the European commission (2002) provided that it is preheated to ensure that it is no longer hazardous to humans. MBM has a composition which makes it interesting as a fertilizer. It typically contains about 50% protein, 10% fat, 8% N, 35% C, 5% P, but small amounts of potassium (K) and sulphur (S) (Hendriks et al 2002; Mondini et al 2008; Brod et al 2012; Möller 2018; Brod et al 2018). The fertilizer effect of MBM to cereals has been reported to be around 80% of the yields obtained with mineral fertilizer (Jeng et al 2004). In Norway, the slaughterhouse industry produces 30,000 Mg MBM, potentially available as fertilizer every year (Haraldsen et al 2011).

Biogas production is a widely used technique for producing energy, and the digestate may be utilized as fertilizer (Nkoa 2014; Möller et al 2008; Möller 2015). Organic materials such as food waste, sewage sludge, fish sludge, macroalgae and animal manure are among the organic resources that potentially can be digested in a biogas reactor. The variability in the biochemical properties of anaerobic digestates is considerable and depends on the input materials (Haraldsen et al 2012; Möller et al 2008; Nkoa 2014). Depending on its biochemical composition, the digestate may be highly valuable as fertilizer, as it contains macro- and micro-nutrients in both organic and inorganic form (Möller and Stinner 2009). However, utilization of the digestate as fertilizer requires proper management and knowledge to avoid negative effects such as greenhouse gas emission, acidification, nutrient losses and contamination with pollutants. In Norway and Europe, there are regulations for the treatment of fertilizer material and permissible contents of pollutants in fertilizer materials and soil amendments (European commission 2016; Norwegian ministry of agriculture and food 2003).

1.3 The nitrogen fertilizer effect of organic materials

The N fertilizer effect of organic materials depends on N mineralization–immobilization and on biogeochemical processes as ammonia volatilization, ammonium fixation, nitrification, denitrification and nitrate leaching. From these processes, the synchronization between the amount of plant-available N and the crops N demand is decisive for the effectiveness of the fertilization (Myers et al 1994). Optimum fertilization management practice should preferably result in low negative impact on the environment without reducing the yield and quality of the produce. Therefore, knowledge about N mineralization patterns from organic materials relevant as fertilizers resources is important for best possible management practice and proper handling of the fertilizer material (Tei et al 2020).

1.3.1 Nitrogen mineralization from organic fertilizer resources

The N mineralization–immobilization turnover from organic materials is closely linked to C turnover, and hence the decomposition of organic matter, in which *microorganisms* (in agricultural soils mainly bacteria and fungi) play a key role. Breakdown of organic materials is a result of catabolic (dissimilatory) and anabolic (assimilatory) metabolism of heterotrophic organisms. Heterotrophic organisms decompose organic materials to assimilate C, N and other nutrients in their biomass, and through fermentation and respiration processes (energy metabolism) to obtain energy for growth and maintenance. This process releases N as ammonium (NH_4^+) and C as carbon dioxide (CO_2) (Fenchel et al 2006). Depending on microbial N demand, the NH_4^+ released (gross N mineralization) may be re-assimilated in microbial biomass (gross N immobilization). The gross N immobilization depends on the microbial N demand as determined by the availability of C for microbial growth and the N:C ratio in the microbial biomass (Fenchel et al 2006). Consequently, net mineralization of N from an organic fertilizer will be positive if the availability of N through its decomposition (gross N mineralization) exceeds that required by the decomposers for their growth (gross N immobilization) and negative (net immobilization) in the opposite case, provided that inorganic N from other sources (e.g., soil and fertilizers) is available. If not, soil inorganic N may be exhausted to the extent that the decomposition rate decreases (Murphy et al 2007). As decomposition proceeds, declining availability of C and energy will eventually limit microbial growth, and sooner or later available N will exceed the demand of the decomposer community, resulting in re-mineralization of some but, usually not all, due to humification and loss processes, of the previously immobilized N. This is schematically illustrated in Figure 1 for a pool of uniform degradability.

The *biochemical and structural quality* and amount of added organic materials are decisive for how much C is available to microbes. Organic materials consist of C and N compounds with different decomposability; some are easily available to microbial decomposers and are readily mineralizable (e.g., amino acids, proteins, soluble compounds), whilst others are more slowly degradable (e.g., hemicellulose-, cellulose- and lignin-like substances).

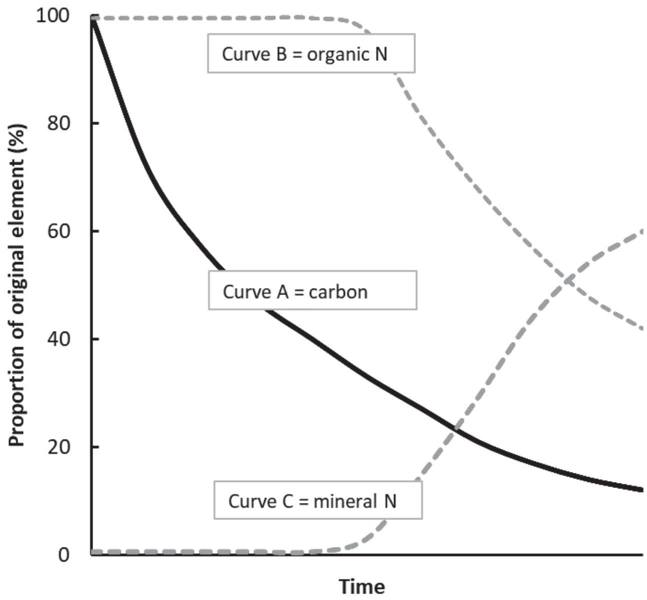

Figure 1 . A schematic illustration of the biochemical quality index C:N ratio of added homogenous organic materials (C:N ratio at 100) as a criterion for deciding whether there is a net immobilization or mineralization from organic materials. Curve A= Carbon in organic materials in proportion of original; curve B=proportion of N in organic form; curve C= Mineralized N in proportion of added N. Illustration idea from Swift et al (1979).

The most important environmental factors determining C and N mineralization and immobilization processes are temperature and moisture. In most soils, increasing *temperature* from the freezing point will increase the biological processes exponentially. The curve flattens when the microbial activity is at an optimum. If the temperature is still increasing after the microbial optimum, there will be a negative effect on microbial activity (Roderigo et al 1997). How temperature influences the microbial breakdown of organic materials is often described by the Arrhenius equation (Kirschbaum 1995), which is an exponential function of energy requirement, universal gas constant and temperature. However, this theoretical expression is complex and, therefore, the Q10 factor is commonly used in models to express the influence of temperature on decomposition. Q10 indicates the change of the decay coefficient when the temperature changes by 10°C (Kirschbaum 1995). Soil *moisture* influences many physical processes in soils (e.g., gaseous exchange, diffusion of nutrients and compounds and water movement), which also influence microbial activity. Mineralization increases with increasing moisture. These processes interact with soil texture and structure, porosity, pH and organic matter. Optimum soil pore water potential for N mineralization is between –0.01 and –0.05 MPa, which corresponds to moisture at field capacity or wetter. In most soils, net N mineralization is linearly related to moisture in the available moisture range. Mineralization is strongly inhibited when the soil pore water potential is less than –4.0 MPa (Myers et al 1982), and at saturation (0 MPa). In mechanistic models, these factors are most often considered as independent factors with no interactions under the decomposition of added organic materials. Functions for adjustments of decomposition rate coefficients to soil temperature and soil water pressure potential are used, e.g., in the Daisy model the decomposition rate coefficient at standard conditions (10°C and –0.01 MPa) are multiplied with modifying factors for temperature and moisture. The temperature factor increases from 0 to 4 with increasing temperature from 2 to 30°C. A factor 1 is used for optimum water potential (Hansen et al 1990; Hansen 2002). The complexity of mineralization and immobilization is illustrated in the brown boxes in Figure 2.

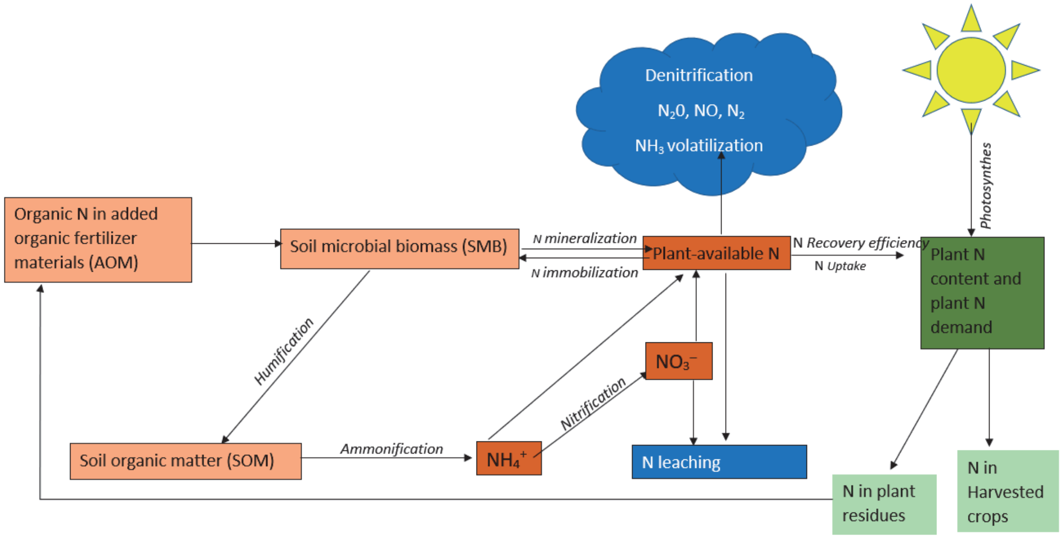

Figure 2 Schematic illustration of the soil nitrogen cycle when adding organic fertilizer materials. The illustration includes N mineralization–immobilization, ammonification, nitrification and loss processes (brown and blue boxes). The crop N demand, uptake, and recovery are illustrated with green boxes.

1.3.2 Synchronization of nitrogen availability with plant demand

Sufficient N is required to ensure optimal vegetable yield. How efficiently the plant-available N is recovered in crops depend on the synchronization of N mineralization with crop requirement (Myers et al 1994). Ideally, N mineralization rate should be slow when crop N demand is small, and fast when the requirement is large. Lack of synchronization may occur when organic N is mineralized after harvest or when mineralization is larger or smaller than plant uptake during the growing season. A schematic example of the rate of N mineralization from organic fertilizer materials in relation to plant N demand is illustrated in Figure 3. During the period from application until the N mineralization rate fits the plant requirement, it would be desirable to stimulate a temporal immobilization of N by adding organic materials with high C:N ratio as to enhance microbial N immobilization. Remineralization of immobilized N has been studied by Chaves et al (2007), who manipulated N mineralization by adding organic wastes. In vegetable production, an asynchrony between crop demand and N mineralization in the post-harvest period can occur, as many vegetables are harvested when having their highest growth rate, when the N demand is still very high. In the post-harvest period, from harvest to frost, the risk of losing N to the environment is high. The risk of loss is highest where mineral N accumulates in soil before the crops demand N, or in soil with bare fallow and nutrient-rich residues (Myers 1994).

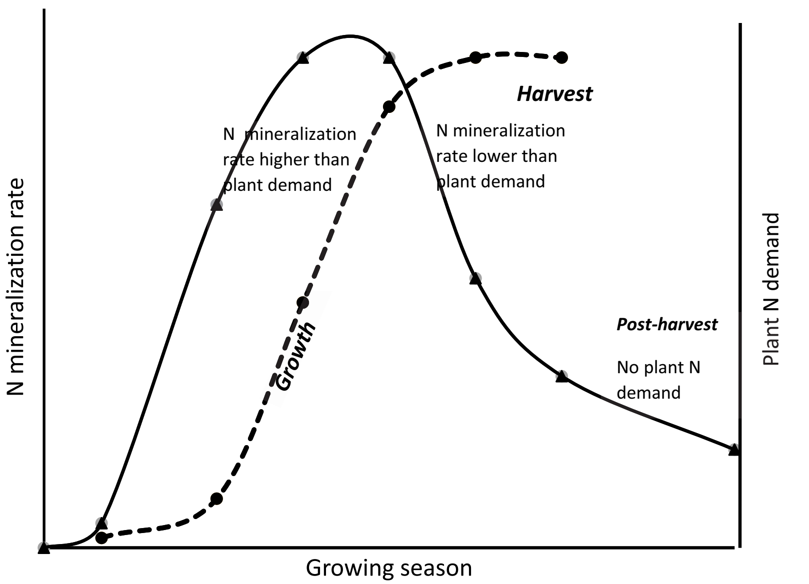

Figure 3. Schematic illustration of accumulated N mineralization from organic fertilizers in relation to crop N demand during different growth stages.

Lack of synchronization between N mineralization and crop demand contributes to a potential risk for losing N to the environment through ammonium fixation, nitrate leaching, ammonia volatilization or denitrification (blue boxes in Figure 2). Nitrate (NO_3^-) is more susceptible to *N leaching* than NH_4^+, which can be adsorbed to clay particles in mineral soils (*ammonium fixation*) (Craswell and Godwin 1984). There is more N leaching in soil with low water-holding capacity, especially during heavy rain or in well-drained soils (Di and Cameron 2002). The potential of leaching N is particularly high when growing crops with shallow roots. To avoid N leaching and increase N recovery in crops, management practices such as precision fertilization, growing cover-crops in bare-soil periods or choosing genotypes and cultivars with deep rooting systems and large N uptake, may be implemented. *Ammonia volatilization* is another pathway for loss of N. The N loss through this pathway is dependent on C:N ratio and the concentration of NH_4^+ (de Ruijter et al 2010; Craswell and Godwin 1984; Cameron et al 2013). Ammonia volatilization increases linearly with increasing N concentration (de Ruijter et al 2010). The risk of losing N as ammonia is high for organic materials with a high proportion of NH_4^+ at application, such as anaerobically digested waste, manure and slurry (de Ruijter et al 2010), especially in combination with high soil pH (Möller 2015), due to chemical reaction between NH_4^+ and hydroxide-ion ($NH_4^+ + OH^- \leftrightarrow NH_3 + H_20$) (Carmeron et al 2013). Moist soil reduces the incidence of ammonia volatilization, hence, application before rainfall or irrigation following fertilization may reduce the loss of N. Soil with high cation exchange capacity stores more NH_4^+. Organic material and residues which decompose on the soil surface lose a larger amount of ammonia compared to incorporated material. *Denitrification* occurs in anaerobic soils when heterotrophic microorganisms (denitrifying bacteria) use NO_3^- instead of O_2 as electron acceptor during respiration (Robertson 1989; Robertson and Groffman 2015; Cameron et al 2013). Denitrification increases with increasing pH. The N_2O:N_2 product ratio of denitrification is influenced by soil pH: at low soil pH the N_2O:N_2 ratio is increasing. At pH 6, the amount of each gas is shown to be approximately equal (Sagger et al 2013). Thus, denitrification depends upon the contents of C and nitrate, as well as upon temperature and level of O_2 and the soil pH (Cabrera 1994). Moist soils with low oxygen level and high pH in combination with hotspots of C accelerate denitrification.

1.4 Nitrogen and crop production

N is the most important limiting factor for crop production. Prior to industrial production of mineral fertilizer, the N supply was demanded on natural N fixation and crop rotations. In the "green revolution" during the period from 1960 to 2000 producers were encouraged to use excess level of mineral fertilization in addition to pesticide, intensive irrigation, mechanisation and use of high-yield breeding cultivars. The industrialization of food production resulted in an increase in yield and the ability to meet the increasing food demand of a growing population (Tilman et al 2002). Management practise to maximize the yield by use of high input of N fertilization resulted in low N use efficiency and detrimental effect on the environment. In the end of the 2000 century the issue related to high N fertilization rates was met by focusing on sustainable production systems with low impact on the environment: A balance between environment, yield and quality (Albornoz 2016).

1.4.1 Nitrogen and plant physiology

N is the fourth most abundant element in plants (in addition to C, O, H), and is an essential nutrient for optimal plant growth and development. It plays a key role in several physiological and metabolic processes and is a crucial constituent in amino acids, protein, enzymes, nucleic acids, and hormones (Mengel and Kirkby 2001), and thereby essential building blocks for cell material and plant tissue. N is also important for synthesis of secondary plant metabolites. In plants, the N is assimilated into amino acids, which is combined into protein or nucleic acid. Protein is building block for chloroplasts, mitochondria, and other structures in the cells where the biochemical reactions occurs. The constituents of N in chlorophyll makes it important for photosynthesis (Mengel and Kirkby 2001).

Plants grown with limited supplement of N have low photosynthetic activity and exhibits deficiency symptoms as chlorosis, especially in older leaves. Under severe limited N conditions, the leaves can become completely yellow or die. Younger leaves will stay green longer, as the N is mobile in the plant and can be allocated from older to younger leaves. Plants grown with excess N level is often dark green, has a high photosynthetic activity, a high vegetative growth, an abundance of leaves and a reduced root system giving a high shoot:root-ratio (Mengel and Kirkby 2001).

1.4.2 Nitrogen uptake, use and recovery efficiency in plants

N may be taken up by plants in cation or anion form: ammonium (NH_4^+) or nitrate ($NO_3^–$). Uptake of $NO_3^–$ is mainly active, which includes a H^+/ $NO_3^–$ cotransport. The H^+ pumped out of the cell as the $NO_3^–$ enters the membrane, is recycled into the cytosol. Therefore, $NO_3^–$ uptake will increase the pH level in the soil. The uptake of NH_4^+ is mainly passive, driven by different electropotensial gradients and cation selective channels. The uptake of NH_4^+ is optimal under pH neutrial soil, and is depressed as the soil acidity is increasing. The uptake of NH_4^+ will increase the acidity of the soil as H^+ is being exchanged by the root under uptake, and not recycled back into the cytosol as under uptake of $NO_3^–$. Whether the plant takes up N as NH_4^+ or $NO_3^–$ depends on the availability of the two N forms. The most common uptake form is $NO_3^–$ as NH_4^+ forms are fast transformed to $NO_3^–$ during the nitrification process and due to agricultural N fertilizers are commonly present as $NO_3^–$. NH_4^+ is not as mobile as $NO_3^–$ in the soils as positive charged ions can be fixed to the soil. Uptake of N as a cation (NH_4^+) reduce the uptake of other cations (as K^+, Ca^{2+} and Mg^{2+}), and will enhance uptake of anion (as phosphate $H_2P0_4^–$ and Sulphur $SO_4^{2–}$)(Mengel and Kirkby 2001).

The crop N use efficiency has been defined in different ways, but most definitions is about the ability of a production system to convert N input into output. In vegetable production systems, the N use efficiencies are in general low due to the use of N as a "cheap insurance" for obtaining high yield and economic outcome (Tei et al 2020). Generally, less than 50% of N supply as fertilizer is not been utilized by crops (Raun and Johnson 1999; Garnett et al 2009). The short-term N use efficiency response on the crops can be calculated in different ways. Most commonly N use efficiency is expressed as a simple index for economic yield, uptake or utilization: Agronomic efficiency, physiological efficiency and recovery efficiency (Craswell and Godwin 1984). *Agronomic efficiency* is the yield ratio per kg N supply and *physiological efficiency* is the ratio of yield per kg N in crop. The fractions of fertilized N taken up by crops is *apparent N Recovery efficiency*, and are defined by Greenwood (1989) and Craswell and Godwin (1984):

$$NRE = (N\ UPTAKE_f – N\ UPTAKE_0)/N_f$$

Where N $UPTAKE_f$ is the total N taken up in fertilized above-ground biomass per unit area and N $UPTAKE_0$ is the N uptake in unfertilized above-ground biomass per unit area, and N_f is the amount of N fertilization per unit area. The fraction of fertilized N taken up by plants is decreasing with increasing fertilization rate, thus, the lower N fertilization the higher apparent

N recovery efficiency. The main challenge is to reduce the quantity of N application without reducing the quality and to keep the yield reduction to a minimum level. The highest possible N recovery efficiency without reducing the yield and quality, which is a compromise between environment, yield and quality.

The N use efficiency is a complex task and is governed by multiple factors. The N use efficiency from organic materials depends on the N mineralization and amount of plant-available form of N and the synchronization with plant N demand, as describe in paragraph 1.3.2 *synchronization of plant demand with available N*. The crops N demand and *growth rate* is the most important factors for regulating the N uptake. The crop N uptake and growth may also be limited by imbalance of other nutrients in the fertilizer material, according to Liebig's *law of the minimum* (Havlin et al 2005; Brod et al 2018; Möller et al 2018). How efficient the production system uses the available N is impacted by management practice, weather conditions, physical and chemical soil factors (Myers 1994). The choice of *Species* and *genotype* are also important for increasing the N use efficiency. This aspect of the N use efficiency has been recognized as the "second green revolution", which aim to identify plant gen that are important under N biosynthesis in plant and which can improve the use effectiveness of N in plants (Palme et al 2014).

1.4.3 Crop growth and nitrogen requirement

In general, vegetables have a high N requirement (Feller and Fink 2005). Crops N demand depend on growth rate and growth curve (van Oosterom et al 2009). The different developmental stages of the plants, requires different levels of N (Figure 3). Therefore, the crop N uptake is regulated by the plant growth itself. *Crop growth* is affected by many abiotic and biotic factors which influences the physiology and photosynthesis of the crops, and can be divided into genotypic (e.g. roots, species), managemental (e.g. nutrition, soil, competition between plants, plant density, shading, water, management practice, pathogen, herbivore) and environmental (e.g. climate, sun light, temperature, geographical locations) factors (green boxes in Figure 2) (Greenwood 1982; Myers et al 1994). Due to seasonal and climatic variation, the growth rate and yield potential vary between years, thus, the N requirement for receiving the maximum yield also varies. These uncertainties and seasonal variations are often the reasons for N fertilization being in excess of requirements, in order to ensure high yield.

The growth of crops can be divided into vegetative and reproductive phases (Mengel and Kirkby 2001). In the vegetative growth phase crops produce leaves, shoot, steams, and roots.

The vegetative growth phase is responsible for biomass production from photosynthesis products and nutrients. The crops capture CO_2 from the atmosphere and transform it into C compounds through the photosynthesis, and the roots take up nutrients and water from the soil. The vegetative growth phase is the basis for yield production through the N containing photosynthesis product protein, amino acid, and nucleic acid. N is often considered to be the most important limiting factor after water deficiency for biomass production, as it influences the vegetative growth rate to a large extent. Many vegetables are harvested during this vegetative growth phase when the N demand is at the highest level. The C compounds and nutrients from the vegetative phase are the source for developing the storage or reproductive organs in the reproductive growth phase (Gastal and Lemaire 2002)

The highest N requirements and most of the N uptake occurs in the vegetative phase. During the vegetative growth phase, the plant N concentration declines as the plant grow and mature (Greenwood 1982; Greenwoods et al 1986) due to a decline in leaf area per unit of plant mass (structural), plant aging and because of remobilization of N from older to new leaves. The ratio of structural tissues (cell walls and storage tissues) in relation to metabolic and photosynthetic tissues increases as the plant grows. As N is primarily located in the cytoplasm and photosynthetic tissues (with less N located in structural tissues), the plant N demand decreases per unit plant mass (Greenwood 1982). This decline in plant N concentration can be described by different mathematical equations. The decline in N concentration in relation to dry matter accumulation is described by a "dilution curve" with the following equation (Lemaire et al 1985):

$$N\% = aW^{-b} \qquad \text{(Equation 1)}$$

Where *W* is the dry matter in megagram per hectare, coefficient *a* is the plant N concentration when the biomass is 1 Megagram per hectare and coefficient *b* is dimensionless. Under low N conditions the growth rates are depressed as the leaf area will be lower, as a consequence of lower cell division and leaf expansion rates. This again leads to reduced the radiation use efficiency due to a lower leaf area for photosynthesis activity. This indicated the importance of leaf area for growth rate (Lemaire et al 2019).

Greenwood et al (1990; 1991) defined a critical N concentration which is the minimum plant N concentration for maximum growth rates. The critical N concentration is a relationship between plant biomass and plant uptake when the N is not a limiting factor for growth. The dilution curve (equation 2) was defined for critical N concentration in crops:

Critical N concentration = acW^{-b} (Equation 2)

Where *ac* is the critical N concentration in plant when *W* is 1 Megagram per hectare. There are crop specific curves for critical N concentration in plant for optimal growth: lettuce (Conversa and Elia 2019), cabbage (Ekbladh and Witter 2010), broccoli and cauliflower (Conversa et al 2019; Riley and Vågen 2003) and Potato (Greenwood et al 1990; 1996). An equation (equation 3) which applies to many crops was described by Greenwood et al (1986):

critical %N= $1.35(1+3^{-0.26W})$ (Equation 3)

The critical N concentration curves can be used to calculate the N nutrition index (NNI) which is the ratio between the actual amount of N in crop and the critical N concentration. The index is a prediction tool for diagnosing the nutrition status and determining the yield at an early plant growth stage (Lemaire et al 2008).

Crop simulation models include mathematical equations to estimate the crop's N requirements. In most dynamic models, crops N demand is expressed as N concentration in above-ground biomass during the growth period, expressed as maximum, minimum and critical %N concentration in crops as a function of time. Other variables in the equation for different crops were later defined and used in N models (Rahn et al 2010; Greenwood et al 2001).

1.4.4 Nitrogen and quality of vegetables

The quality of horticulture products can be divided into internal and external quality (Schreiner et al 2013). External quality is associated with parameters like size, colour, shape, and disorders (Stefanelli et al 2010). These external quality parameters are important for purchasing decisions and give consumers their first impression of the quality of the product. Internal quality parameters are not visible to the consumer, and include flavour, taste, contents of macro- and micro-nutrients, possible hazards (e.g., nitrate, pesticide residues, mycotoxins, faecal bacteria), pollutants (heavy metals and other environmental poisons), secondary metabolites and health-related compounds (e.g., glucosinolates, phenolic compounds, carotenoids, and ascorbic acid) (Verkerk et al 2009; Schreiner et al 2013; Rembialkowska 2007). Vegetable quality is complex, including both physiological attributes and consumers preferences and meanings.

1.4.4.1 Nitrogen fertilisation and external quality of vegetable crops

N is important for optimal growth and development of plants as described in paragraph *1.3.1 Nitrogen and plant physiology*. N deficiency symptoms in vegetables is well documented (Mengel and Kirkby 2001). Attributes as color, form and size are affected by N fertilization. These attributes are related to the N's constituents in protein and chloroplast, as well as the impact on cell volume (Stafanelli et al 2010; Mengel and Kirkby 2001). In general, low N fertilization results in poor growth, low yield, pale green color and small sized crops, and high N fertilization is associated with darker green, greater size and higher yield. High N fertilization rates are associated with vegetative growth rate at the expense of root growth and generative growth (Mengel and Kirkby 2001). Root growth and root braching is restricted with high N fertilization, which might result in lower yield for potato. Low N fertilization in leafy vegetables as lettuce results in yellowish or pale leaves, and occurs first in the older leaves. In head-forming vegetables, the head shows uniform paling, small and loose heads, and there is a risk for bolting for broccoli grown under low N availability. Split head in head forming vegetables can be related to high N fertilization rates (Locascio et al 1984). The last decades, impact of excess N fertilization on vegetable crop quality has gained attention (Stefanelli et al 2010; Albornoz et al 2016). Excess N fertilization may influence the quality negatively, however, the impact of N fertilization on the *external* quality are rather low (Locascio et al 1984). Shelf-life and susceptibility to pathogen and disorders during storage are also related to high N fertilization (Mengel and Kirkby 2001; Locascio et al 1984).

1.4.4.2 Nitrogen and Internal quality of vegetable crops

1.4.4.2.1 Sensory quality

Nitrogen application rates and form might influence the sensory quality of vegetables, e.g., taste of swede (Thomsen et al 2018), sugar content in carrot (Smolen and Sady 2009), and sugar and drymatter in other vegetable crops (Bourn and Prescott 2002). However, the effect of N fertilization on the sensory and taste evaluations of vegetables show inconsistent results. Many research studies have compared the sensory quality of conventional compared organic produced vegetables, which is assosiated with a lower availability of plant-available N. For example, potato from organic farms have obtained better sensory evaluation than potato and carrots from conventional farms (Rembialkowska 2003). However, the general conclusion is that there are no convincing evidence that organic vegetables are more tasty than conventional (Bourn and Prescott 2002).

1.4.4.2.2 Nitrate accumulation in food crops

Nitrogen fertilisation may, in some situations, cause an accumulation of high levels of nitrate (NO_3^-), which may negatively impact consumer health. It is not NO_3^- itself, which gives the negative health effect but is related to the synthesis of toxic nitrite and nitrosamine compounds in the body (Santamaria 2006; Jones et al, 2015). The nitrite may cause cardiovascular diseases and cancers and has high toxicity to infants. The level of N fertilization and management practice can impact the NO_3^- content in vegetables (Konstantopoulou et al 2010; Santamaria 2006; Albornoz 2016). Crops accumulate more NO_3^- when N fertilization increase. Under limiting N availability in soil (reduced fertilization levels), the NO_3^- accumulation decreases (Santamaria 1998). The timing and rate of application and the N fertilization form (NH_4^+-N or NO_3^--N) affects the content of NO_3^- in vegetables (Santamaria et al 2001). Organic management practice gives in general lower NO_3^- content in vegetables than conventional (Raupp 1996). NO_3^- accumulation and assimilation in vegetable crops are also dependent on the genetic factor (species and variety) and environmental factors (light and temperature). High N fertilization promotes the accumulation of NO_3^- in plant tissues due of excess N uptake during growth. When taken up in excess amount, the NO_3^- is stored in the vacuoles for later assimilation, reduction to NH_4^+ for protein synthesis or for use in other N compounds.

The content of NO_3^- in various plant part differ (Santamaria 1999). The highest level of NO_3^- is in the leaf, steam, and root, and lowest in the seeds and fruit. Especially in vegetables belonging to the families *Brassicaceae* (e.g., cabbage, broccoli, cauliflower), *Chenopodiaceae* (e.g., beetroot, spinach), *Apiaceae* (e.g., carrot, parsley) and *Asteraceae* (e.g., lettuce, endive, leafy chicory) the NO_3^- accumulation may be high, whereas, in *Solanaceae* (potato) and *Liliaceae* (e.g., garlic, onion) accumulation is low (Santamaria et al 1999). The health concern related to NO_3^- intake is highest for leafy vegetables due to the high average consumption per meal. Lettuce is one of the vegetables that contribute most to daily NO_3^- intake (Santamaria et al 1999).

1.4.4.2.3 Glucosinolates and other secondary metabolic compounds

Secondary metabolites are part of the plants' defence mechanism to abiotic stress, herbivore and pathogens. Polyphenols, vitamin C, carotenoids and glucosinolates are secondary metabolites that are found in fruit and vegetables. Stress conditions as suboptimal growth conditions for the crops, such as an insufficient supply of N or the presence of insect herbivores, may influence the synthesis of secondary plant metabolites (Bourn and Prescott 2002; Young et al 2005). This can partly be explained by the C:N balance theory (Bryant et al 1983; Coley et al 1985; Brandt and Mølgard 2001; Rembialkowska, 2007). The C:N balance theory states that with an excess level of plant-available N, compounds with high N contents are synthesized (e.g., amino acids, proteins and N-containing secondary metabolites such as alkaloids), and when the N becomes limited, the metabolism in the plants will turn toward more C-containing compounds (e.g., cellulose, starch and secondary metabolites with low N content such as phenolics). Under high N fertilization, the growth and photosynthesis rates are high, at the expense of synthesis of C based secondary metabolites. In the opposite case with low N availability, growth rate and photosynthesis are low, thus, C containing metabolites are synthesised.

Glucosinolates is the main class of secondary plant metabolites found in the *Brassicaceae.* In broccoli,16 glucosinolates have been identified (Vallejo et al 2002; Vallejo et al 2003; Latte et al 2011). Based on the amino acid they originate from, glucosinolates can be divided into aliphatic (major compounds are glucoraphanin and glucoiberin), indolic (major compounds are glucobrassicin and neoglucobrassisin) and aromatic glucosinolates (Meyer and Adam 2008; Vallejo et al 2003;Vallejo et al 2002). Aliphatic glucosinolates are derived from methionine, isoleucine, leucine or valine, indolic glucosinolates obtain from tryptophan and aromatic glucosinolates from phenylalanine or tyrosine. All glucosinolates are based on glycopyrano connected to O-sulphated thiohydroximate (Rollin and Tatibouët 2011); which involve N and S in the chemical structure. The structures of the main individual glucosinolates found in broccoli are illustrated in Figure 4.

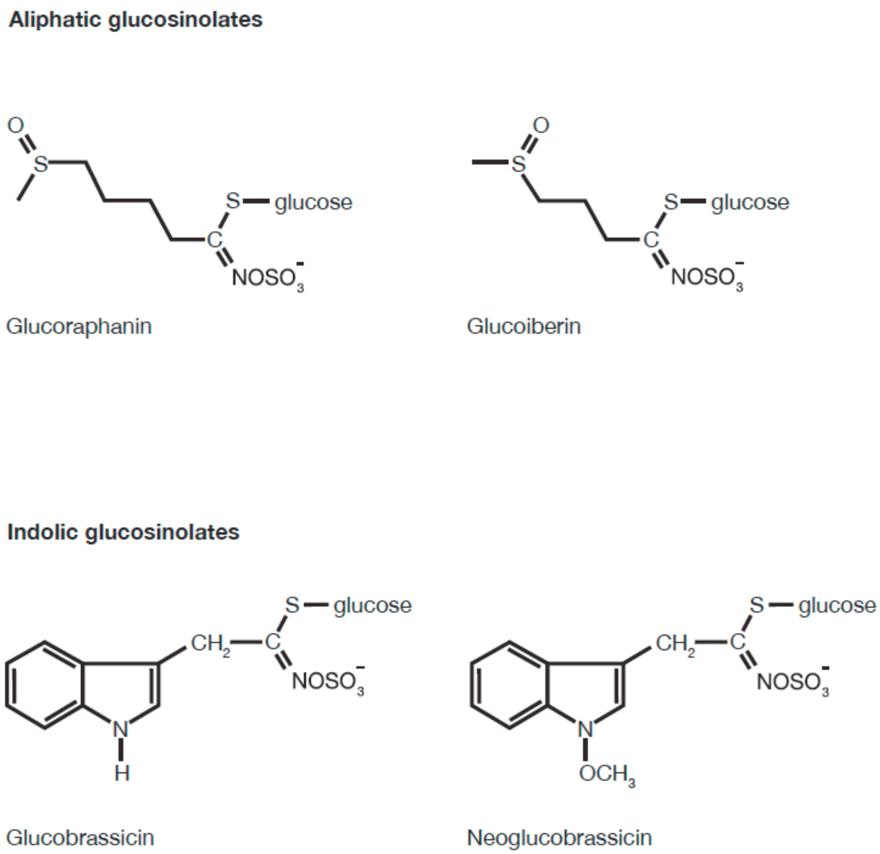

Figure 4 Chemical structure of the main individual glucosinolates found in broccoli (*Brassica Oleracea* var. *italica*). The upper two structures are aliphatic glucosinolates (Glucoraphanin and Glucoiberin), and the two lower structures are indolic glucosinolates (Glucobrassicin and neoglucobrassicin).

The level and combinations of glucosinolates in the crop depends on many interacting factors genetic, cultivar, abiotic (climatic and environment) and agronomic factors (Vallejo et al 2003). Nutrient availability to crops is shown to impact the amount and type of glucosinolate compounds. The level of glucosionlates and their hydrolysis products (e.g. sulforaphane, which is an anti-cancer product in broccoli) is related to fertilization. Nitrogen and Sulphur (S) fertilization and the relationship between these nutrients influence the total content of glucosinolates and individual glucosinolates. Li et al (2007) showed that the total glucosinolate level did not respond to increasing N fertilization at high S fertilization level except for an increase in N-containing tryptophan-derived indolic glucosinolates. However, low S fertilization level result in an increase in the methionine-derived aromatic and aliphatic glucosinolates decreased with an increasing N fertilization. Also Schonhof et al (2007) found a relationship between N and S fertilization on the content of glucosinolates: at insufficient N supply, an increase in total glucosinolate was independent of S fertilization, but at insufficient S and optimal N supply the total glucosinolate level decreased. The total glucosinolate level and level of individual glucosinolate (glucoraphanin, sinigrin, glucobrassisin, gluconapin and progoitrin) increase with increasing S fertilization (Krumbein et al 2001; Kaur et al 1990). Meyer and Adam (2008) showed a higher content of the indolic glucosinolate glucoprassision and neoglucobrassisin in organic broccoli compared to conventional broccoli.

Other secondary metabolites as polyphenol, carotenoid and vitamin C in vegetables are shown to be influenced by nitrogen fertilization. *Polyphenol*s are secondary metabolites found in fruits and vegetables. Polyphenol can be divided into 16 classes, and the four main classes are phenolic acid, flavonoids, tannins and chalcones & Coumarins (Giada 2013). All polyphenols have a chemical structure including one aromatic ring, at least one hydroxyl group and commonly bound to other molecules (Giada 2013). The influence of N fertilization on phenolic compounds, which are mainly C-based secondary metabolites, has been investigated in several research studies (Bryant et al 1983; Sousa et al 2008; Hamouz et al 2006; Koh et al 2012). In most cases, a negative relationship between high N fertilization and contents of total polyphenols has been observed (Stefanelli et al 2010). For broccoli, flavonoid content were found to decrease with increasing N level (Fortier et al 2010; Becker et al 2015). N fertilization amount and N form and application method (foliar application) have shown to influence the polyphenol content (Sady et al 2010; Smolen and Sady 2009). The contents of polyphenols is shown to be higher in organic compared to conventional cabbage (*Brassica oleracea* var. *capitata*) (Sousa et al 2005), broccoli (*Brassica oleracea* var. *italica*), potato (*Solanum tuberosum)* (Hamouz et al 2006) and spinate (*Spinacea oleracea*) (Koh et al 2012). *Vitamin C* is the most important vitamin in vegetables (Lee and Kader 2000). Vitamin C is consideres as the sum of ascorbic acid and dehydroascorbic acid. The latter is the oxidized form of ascorbate. As reviewed by Lee and Kader (2000), N fertilization influence vitamin C content in vegetable crops positive (Muller and Hippe 1987) and negative (Sorensen et al 1994; Mozafar 1993). In general, the vitamin C content is increasing with decreasing N fertilization, which is explained by higher growth rate and a dilution effect.

However, if the N level is suboptimal, the synthesis of Vitamin C dropps. This indicates that vegetable crops demand a certain amount of N for Vitamin C synthesis (Mozafar 1993).

1.5 Modelling as a tool for predicting nitrogen dynamics in crop production

Mathematical models are tools which imitate the reality and are useful for understanding the turnover dynamics of C and N from applied organic materials. There are two basic dynamic models: empirical and mechanistic. *Empirical models* are simple relationships among measured data. This includes simple equations and curves to estimate N yield responses and environmental impacts, e.g., based on C:N ratio, which is commonly used as an indicator to determine the decomposition of plant residues and N mineralization (Nicolardot et al 2001). Empirical models have been established in the form of quantitative relationships between different biochemical quality indices (total N, lignin, cellulose, hemicellulose, polyphenol and C:N ratio) of organic materials added to soils and N mineralization (Vigil and Kissel 1991; Heal et al 1997). Such static models are useful to have an idea about net N mineralization but unable to capture the temporal C and N turnover dynamics along the decomposition continuum as described above and as influenced by environmental factors such as soil temperature, moisture, texture, structure and pH. For this, *mechanistic models*, i.e., models based on known mechanisms and including the temporal dimension, are needed. Mechanistic N models are more comprehensive imitations of reality. Mechanistic models that simulate N dynamics are useful tools to improve the understanding of the complex processes going on in the soil during decomposition of organic materials (Di and Cameron 2002). Properly calibrated and validated soil–plant–atmosphere models, may help scientists and agricultural advisers to predict the N fertilizer effects of organic materials on crop biomass, quality and marketable yield, and impacts on the environment. These models attempt to estimate responses of a complex of processes such as biogeochemical processes in soils and crop growth. In such models, organic fertilizer materials are traditionally partitioned into pools or fractions each assumed to have uniform degradability. The pools are based on potential decay of labile or stable degradable biochemical substrates (Rahn et al 2010; Molina et al 1983; Verberne et al 1990; Hansen et al 1990; Johnson et al 1987). Some models handle organic material as one pool (APSIM, Probert et al 1998) whereas other divide into two (e.g., CENTURY, Parton et al 1987), or three pools (DAISY, Hansen et al 1990; SOILN, Johnson et al 1987; EU-Rotate_N, Rahn et al 2010). Approaches for partitioning the plant residue C and N into pools have been discussed by e.g., Borgen et al (2010). Common methods for determining chemical composition as related to degradability of organic materials are Near Infrared Radiation (NIR; Henriksen et al 2007) and stepwise chemical digestion (Goering and van Soest 1970). Pools can also be determined by inverse parameterisation estimation by fitting the fraction parameter to C and N mineralization data obtained under controlled temperature and moisture conditions (Breland and Eltun 1999).

One model developed to predict yield, environmental impact and economic profit in vegetable production is the EU-Rotate_N model. This model is a mechanistic model developed to assess the economic and environmental performance of N fertilization and rotational practices (Rahn et al 2010). The model consists of modules for N mineralization, N uptake and crop growth, as well as separate modules for root growth, water, snow and frost, soil fertility building, and marketable yield. The model has been tested in field studies in parts of Europe (Rahn et al 2010; Doltra and Munoz 2010; Nendel et al 2013, Suarez-Rey et al 2016) as well as in greenhouses (Guo et al 2010; Sun et al 2012; Soto et al 2014). The calculation of N mineralization from organic matter in EU-Rotate_N is based on the routines used in the DAISY model (Hansen et al 1990). The mineralization module predicts N release from soil and traditional organic fertilizers such as animal and green manures, but not from organic N sources from the food industry. Thus, the model has a potential to be further developed for locally available organic resources relevant for both organic and conventional vegetable production.

The EU-Rotate_N model operates with a daily interval, and its modules are driven by input data on the biochemical quality of added organic matter (AOM), as well as climatic conditions (temperature, rainfall) and physical and chemical properties of the soil. The C and N turnover in the soil involve three main pools: AOM, soil microbial biomass (SMB) and soil organic matter (SOM). Each pool is divided into two sub-pools with slow (AOMs, SMBs and SOMs) and fast (AOMf, SMBf and SOMf) decomposition rates, respectively. The decomposition follows first-order kinetics:

$$dC_x/dt = k_x C_x \qquad \text{(equation 1)}$$

where dC_x/dt is the turnover rate (kg C ha^{-1} day^{-1}) of pool x (AOM, SMB or SOM pools), C_x is the content of C in pool x at time t and k is the first-order decomposition rate coefficient (decay rate constant), which is fixed for each pool (Hansen et al 1990). In the original version of EU-Rotate_N, C:N ratio and k parameters for crop residues were derived from results of a comprehensive experiment where biochemical quality was determined by stepwise chemical digestion (Jensen et al 2005). Manure and slurry parameters are taken from the DAISY model. The decomposition rate constants are multiplied by rate-modifying coefficients for soil temperature and moisture. In organic materials where decomposition has already taken place, 10% of the C is not divided into slow and fast pools, but considered to converted to humic substances by the humification process. The N pools are calculated from the actual amounts of C in the pools, using a fixed C:N ratio for the pool AOMs:

$$N_t = C_t * N/C \qquad \text{(equation 2)}$$

where N_t is the amount of N in the actual pool at time t, C_t is the amount of C in the same pool at that time, and N/C is the reciprocal of C:N ratio in the respective pool. The daily loss of N from each pool is then proportional to the turnover of organic C as governed by the C:N ratios of the pools.

1.6 Research questions and objectives of the present study

The overall aim of the present study was to determine the potential of organic resources as fertilizers for vegetables measured as yield, N use efficiency and selected product quality parameters.

Research questions (RQ) in this thesis are:

RQ1: What is the potential for N mineralization during decomposition of the selected organic fertilizer materials?

RQ2: Which N fertilizer effect of the organic fertilizer resources, measured as yield and N use efficiency, can be obtained for vegetables under field conditions?

RQ3: How do the organic fertilizers influence vegetable quality?

RQ4: How well can the EU-Rotate N model predict the yield and N parameters of vegetables fertilized with organic materials?

Specific objectives and hypotheses were:

- To investigate the C and N mineralization dynamics of organic resources potentially relevant as fertilizer at controlled temperature and moisture (**Papers III** and **IV**).

    - Hypothesis H1: The C and N mineralisation patterns of organic resources relevant as fertilizer differ widely as a function of biochemical composition of the materials.
- To determine the value of novel organic resources as fertilizers with respect to N use efficiency and vegetable crop yield level (**Papers II** and **IV**)
    - Hypothesis H2: The N use efficiency and yield response of vegetables differ widely as a function of N mineralization from the organic fertilizer resources.
- To investigate effects of fertilization with organic resources on selected quality parameters of vegetables (**Papers I** and **II**)
    - Hypothesis H3: The tested organic fertilizers can influence the sensory quality and content of biochemical compounds in vegetables.
- To enable the EU-Rotate_N model to describe C and N mineralization from the novel organic fertilizer resources under controlled temperature and moisture conditions and to evaluate the model's ability to predict results from a field experiment (**Paper III**)
    - Hypothesis H4a: The EU-Rotate_N model can describe C and N mineralization dynamics of selected organic materials under controlled temperature and moisture conditions
    - Hypothesis H4b: The EU-Rotate_N model can predict yield obtained by use of the selected organic fertilizers.

2. MATERIALS AND METHODS

To predict the C and N mineralization patterns of nine organic resources, incubation experiments were conducted at controlled temperature and moisture conditions. Four of the organic fertilizer resources were selected for field experiments (2008, 2009 and 2010) with broccoli, potato and lettuce in rotation at two locations (Bodø, 67°N, and Grimstad, 58°N), where effects on yield, N use efficiency and selected quality parameters were determined. Finally, data from the incubation were used to calibrate the EU-Rotate_N model, and data from the field experiment conducted at Bodø were used to evaluate the model performance under field conditions.

2.1 The organic fertilizer resources

Organic fertilizer resources were selected for local availability and their potential for recycling nutrients. Four different groups of organic materials relevant as fertilizer were investigated: high-N organic waste of industrial origin, seaweed (algal meal), anaerobically digested food waste and sheep manure. These materials differ widely in their chemical composition and physical properties (Tables 1 and 2). For further details about chemical analysis and handling of the organic materials, see **Papers III** and **IV**.

Table 1 Chemical composition of the organic fertilizer resources. Abbreviations: TOC, total organic carbon; TKN, total Kjeldahl nitrogen; NH_4^+-N, ammonium-N; NO_3^--N, nitrate-N.

Organic resources	pH	DM (%)	TOC (g kg^{-1} DM)	TKN (g kg^{-1} DM)	NH_4^+-N (g kg^{-1} DM)	NO_3^--N (g kg^{-1} DM)	C:N ratio
Shrimp shell pellets **(SSP)**	9.2	91.8	288	71.0	0.3	<0.1	4
Shrimp shell powder **(SSM)**	9.4	93.2	297	73.4	6.5	<0.1	4
Commercial algal meal **(AM)**	6.0	89.5	336	12.0	0.1	<0.1	28
Algal meal *Laminaria digitata* **(LD)**	6.4	90.3	338	18.3	0.1	0.3	19
Algal meal *Saccharina latissima* **(SL)**	6.4	90.5	342	22.2	0.3	0.8	15
Fish sludge waste **(FW)**	5.7	86.0	450	69.0	2.6	<0.1	7
Meat bone meal **(MBM)**	6.5	94.2	432	91.6	0.4	<0.1	5
Anaerobically digested food waste **(AD)**	8.6	0.9	286	676.0	619.0	<0.1	0.5
Sheep manure **(SM)**	8.8	15.0	336	33.7	8.0	<0.1	10

Table 2 Origin and physical properties of the organic fertilizer resources

Organic resources	Physical properties and origin/producer
Shrimp shell pellets **(SSP)**	Pelletized shrimp shell powder produced by Nofima, Bergen, Norway,
Shrimp shell powder **(SSM)**	Shrimp shell powder produced by Bioprawns AS, Nord-Leangen,
Commercial algal meal **(AM)**	A commercial algal meal product from Nordtang AS (Vestbygd, Norway), consisting mainly of the algae species *Ascophyllum nodosum*.
Algal meal *Laminaria digitata* **(LD)**	Collected from the shelf of the North Sea close to Bodø, washed, dried and ground.
Algal meal *Saccharina latissima* **(SL)**	Collected from the shelf of the North Sea close to Bodø, washed, dried and ground.
Fish sludge waste **(FW)**	Fish sludge waste collected from an on-land salmon hatchery, Åsen settefisk AS, Levanger, Norway.
Meat bone meal **(MBM)**	Meal produced by Norsk Protein AS, Mosvik, Norway.
Anaerobically digested food waste **(AD)**	Anaerobically digested household waste from the HRA biogas plant, using technology produced by BioTek AS.
Sheep manure **(SM)**	SM was from NIBIO Tjøtta, Norway.

2.2 Carbon and nitrogen mineralization from the organic fertilizer resources at controlled temperature and moisture: incubation experiments

The C and N mineralization patterns from the selected organic materials were determined by incubation in a dark brown sandy soil collected at Vågønes, NIBIO, Division Bodø. Two different incubation experiments were conducted to determine the C and N mineralization pattern from the organic materials, further in the text referred to *Incubation A* and *Incubation B*. The incubations are described in detail in **Papers III** (*Incubation B*) and **IV** (*Incubation A*). Net N mineralization and emission of nitrous oxide from shrimp shell pellets and powder are published in **Paper IV.** Commercial algal meal, algal meal of *Laminaria digitata* and *Saccharina latissima*, meat bone meal, anaerobically digested and sheep manure were incubated in the same experiment, however, not presented in **Paper IV.** Briefly, for *Incubation A* organic materials corresponding to 0.11 g N kg^{-1} DM soil (corresponding to 300 kg N ha^{-1}, considering a 0.2 m plough layer) were incorporated in 100 g DM soil in 0.2 L open glass jars. The samples were incubated at 15°C and controlled moisture (25 g water in 100 g DM soil) for 100 days. The field capacity for this soil was determined to be 30% by Haraldsen and Grønlund

(1989). The moisture content was checked and adjusted twice a week. At day 1, 14, 21, 69 and 100, three samples were taken from the incubation chamber and stored at −18°C prior to analysis of inorganic N (NH_4^+ and NO_3^-) at NIBIO Apelsvoll. At day 0, 5, 15, 35, 72 and 100, glass jars were sealed with a lid for one hour and gas samples then removed by crimp-sealed serum vials connected to the glass jar headspace (trough a silicon plug in the lid). Samples were analysed for nitrous gas emission by gas chromatography at NMBU according to a method developed by Molstad et al (2010)[1]. Carbon mineralization (CO_2) was analyzed by a Li-8100 gas analyzer (Li-Cor Biosciences UK Ltd, United Kingdom). Due to technical issues, the incubation was repeated (*Incubation B*) to get C and N mineralization data which are related **(Paper III**).

In *Incubation B*, the organic fertilizer materials presented in Tables 1 and 2, equivalent to 380 kg N ha^{-1} (considering a 0.2 m plow layer; 0.14 g N kg^{-1} DM soil), were thoroughly mixed with 50 g DM soil. Soil without fertilizer served as control. The samples were incubated at 15°C for 60 days at constant moisture (a water tension corresponding to 50% of field capacity at 5 kPa). Triplicate cups were destructively sampled at days 1, 10, 18, 39 and 60, stored at −18°C and analyzed for inorganic N (NH_4^+ and NO_3^-) at NIBIO Apelsvoll.

To determine C mineralization, triplicate samples from each treatment were placed in sealed 2 L glass jars equipped with alkali traps for capturing evolved CO_2. The alkali traps consisted of 5 ml 1 M NaOH in 20 ml liquid scintillation vials. These alkali traps were removed, sealed and replaced by fresh ones at day numbers 3, 7, 12, 19, 27, 38, 43 and 60. The C contents of the alkali solutions were analyzed by mixing Na_2CO_3 with concentrated sulphuric acid (3 M H_2SO_4) in a closed mixing cell filled with glass beads, and extracting the evolving CO_2 in a stream of argon (Ar), which was flushed to an infrared gas analyzer (IRGA).

2.3 Effects of organic fertilizer resources on yield and N use efficiency in field experiments

The experimental fields were located at Vågønes at NIBIO, Division Bodø (Northern Norway, 67°28’N, 14°45’E) and Division Landvik, Grimstad (Southern Norway, 58°34’N, 8°52’E)

[1] In Paper IV, reference and description of the method for nitrous gas analysis, as well as information about where the analyses was conducted, were by mistake omitted.

during the growing seasons of 2008, 2009 and 2010. In Bodø, the soil was a sandy orthic humo-ferric podzol, whereas the soil in Grimstad was a gleyed sombric brunisol with southwest-facing slopes of 2−4 and 2−6%, respectively. In the year prior to the experiments, the fields were ploughed (20−30 cm depth) in late July and harrowed (5−10 cm depth) twice (early August and late September) to reduce weeds. Chemical properties of the soils are presented in Table 3, and the meteorological data from the growing seasons 2008, 2009 and 2010 in Table 4.

Table 3. Chemical properties and texture of the upper 0.3 m soil layer of the experimental fields in Bodø and Grimstad (samples collected in spring 2008; TC, total carbon; TN, total nitrogen; NO_3^--N, nitrate-N; NH_4^+-N, ammonium-N; TP, total phosphorous).

	Chemical properties						Texture		
Location	pH (H_20)	TC (g kg^{-1})	TN (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	TP (mg kg^{-1})	Sand	Silt	Clay
Bodø*	6.1	21	1.7	7.0	3.9	840	91	7	2
Grimstad	5.9	30	1.6	11.1	1.2	790	87	10	3

*Corrected soil texture characteristics misrepresented in Paper I

Table 4. Mean day temperature, total precipitation and total sunshine in Bodø and Grimstad for the growing seasons of 2008, 2009 and 2010.

		Mean day temperature (°C)				Total precipitation (mm)				Total sunshine (h)			
Location	year	June	July	Aug	Sept	June	July	Aug	Sept	June	July	Aug	Sept
Bodø	2008	11.3	14.2	12.4	9.5	36	32	29	154	214	211	165	86
	2009	10.5	14.3	14.4	9.6	51	31	107	293	256	201	142	52
	2010	8.7	13.3	12.4	9.7	91	110	51	47	185	161	152	86
Grimstad	2008	14.7	17.3	15.6	11.6	74	101	250	137	-	-	-	-
	2009	14.9	16.8	15.9	13.0	53	244	99	79	276	199	157	-
	2010	15.1	17.0	16.0	11.7	30	68	131	122	278	200	177	-

Factorial field experiments with four of the nine incubated organic fertilizer materials (AD, SSP, SM, AM) were conducted. Each of the materials selected to represent one of the four groups: high-N organic wastes of industrial origin (SSP), seaweed (AM), anaerobically digested food waste (AD) and sheep manure (SM). These materials were supplied at different N application rates in a crop rotation of broccoli (*Brassica Oleracea* L. var. *italic* cv. Marathon) (first-year crop), potato (*Solanum tuberosum* L. cv. Troll) (second-year crop) and lettuce

(*Lactuce sativa* L. cv. Ametist and cv. Argentinas) (third-year crop). Table 5 gives a summary of combinations of fertilizer type and amount. No fertilizer (NF) and mineral fertilizer (MF) given by a combination of NPK 12−4−18 and calcium nitrate (Kalksalpeter) fertilizers (59% of N from NPK), both obtained from Yara (Oslo, Norway), were used on control plots. Potassium sulphate was added to SSP-plots, due to low soil K level. Fertilizer materials were broadcast by hand and incorporated into the soil by a rotary harrow. Broccoli and potato were planted with 18 plants in each row and 4 rows on each sub-plot. The planting distance was 0.33 m, the row space was 0.7 m. In every other row the lettuce cultivars 'Ametyst' and 'Argentinas' were planted on biodegradable film (Orlemans plastic B. V., Genderen, The Netherlands) in beds of four and five rows in Grimstad and Bodø, respectively. Figure 5 shows a picture of the field where the experiment was conducted in Bodø.

Figure 5. Picture of the field in Bodø, where the experiment was conducted. Photo: Ingunn Øvsthus

In the first year of the field experiment, broccoli was planted on biodegradable film based on corn starch (BioAgri, BioBag Norge AS, Askim, Norway) with the aim of reducing leaching and weed growth. Due to problems with dissolution and mineralization of fertilizers in the upper soil layers close to the film cover, this practice was not included in the following years.

Table 5. Type of organic fertilizer resource and application rates (kg N ha^{-1}).

	1st-year crop: broccoli	2nd-year crop: potato	3rd-year crop: lettuce
Fertilizer codes	Fetilizer rates (kg N ha^{-1})		
AD	80	80	0
AD	170	0	60
SSP	80	80	0
SSP	170	0	60
SM	80	80	0
SM	170	0	60
AM	80	80	0
AM	170	0	60
MF	170	80	60
NF	0	0	0

2.3.1 Crop registrations and nitrogen analyses

Broccoli, potato and lettuce were harvested to determine fresh-weight, above-ground dry matter (DM), DM of harvestable yield and N uptake in above-ground biomass. Figure 6 shows the maturation stage of broccoli at harvest. The weight of individual broccoli and lettuce and total weight of potato tubers per plot were measured, and total yield was calculated as the total weight of broccoli heads or potato tubers per unit of harvested area. A selection of lettuce in every other row was harvested. Due to different cultivars, which developed differently, the calculated total yields are an overestimation of expected yield per hectare. To determine the DM and Kjeldahl N, 6–10 complete broccoli plants per plot were harvested and broccoli heads and residues were weighted separately. Potato haulm and tubers of 10 plants were weighed separately. For lettuce, 6–10 plants were weighed. The plant materials were dried at 60°C to determine DM prior to Kjeldahl N analysis at NIBIO Apelsvoll.

Soil samples were collected from two soil depths (0–0.3 and 0.3–0.6 m) in the spring prior to the field experiment (between tillage and planting) and autumn after harvesting. NH_4^+ and NO_3^- were determined by extraction of 40 g soil in 200 ml 1 M KCl and analysis by a Flow Injection Analyser (FIAstar 5000, Foss Analytical AB, Sweden) at NIBIO Apelsvoll.

Disorders and sizes were recorded for all three crops. Detailed description of the registrations can be found in **Papers I** and **II.**

Figure 6. Photo of the maturation stage of Broccoli at harvest. Photo Ingunn Øvsthus.

2.3.2 Calculation of crop nitrogen uptake and apparent nitrogen recovery efficiency

Estimation of N uptake per hectare for broccoli and potato (**Paper III)**, was calculated from DM per hectare. DM for edible parts, was based on the whole experimental plot and DM of residues was calculated out of an average of 6–10 harvested plants (DM_{yield} (kg ha^{-1}) *N% in yield + $DM_{residue}$ (kg ha^{-1}) *N% in residue). For lettuce, estimation of N uptake per hectare was based on an average of the harvested plant.

Apparent N recovery efficiency (NRE) of the fertilizers was calculated as described by Craswell and Godwin (1984).

$$NRE = (U - U_0)/N_A \qquad \text{(Equation 3)}$$

where U and U_0 are uptake of N (kg ha^{-1}) in above-ground plant biomass (including content of N in potato tubers) with and without fertilizer, respectively, and N_A is the amount of N applied (kg ha^{-1}). U_0 is the mean N uptake on the three plots without fertilizer. The method assumes that the N uptake is similar for crops with and without fertilizers. It involves subtracting the N uptake in crops of control plots from the N uptake of fertilized crops.

2.3.3 Health-related components and sensory analyses

Glucosinolate contents in broccoli were analyzed using two methods for determining, respectively, total and individual glucosinolate contents. First, total glucosinolate content in unfertilized broccoli and broccoli fertilized with AD, SSP, SM, AM and MF were analyzed by PlantChem (Klepp, Norway) according to Lange and Lindow (1991). A portion of 0.8–1.6 g fresh-weight of broccoli florets powder, which had been frozen in liquid N and stored at −80°C, was extracted using 3 ml 70% methanol at 80°C for 10 minutes, and then centrifuged. 3 ml palladiumchloride (2 mM $PdCl_2$ in 1 M HCl) was added. The mixture was left at room temperature for 1.5 hours prior to measurements of total glucosinolate contents at spectrophotometer (405 nm). The results were calibrated with a standard sinigrin. Total glucosinolate content was expressed as µmol sinigrin equivalents per gram fresh weight. Based on these results selected samples were analyzed for individual glucosinolates as described in **Paper I**. Briefly, glucosinolate contents were determined for broccoli fertilized with SSP, SM, and MF corresponding to 170 kg N ha^{-1}, and NF. The frozen powder of broccoli florets was freeze-dried (Christ Gamma 1–16, Christ, Osterode, Germany) and ground using a mortar to a fine powder before extraction. Samples for HPLC analysis were prepared according to the method of Vallejo et al (2002) and ISO 9167-1:1992, with several modifications. The analysis was conducted at Nofima AS (Ås, Norway).

For sensory analysis, ten randomly selected broccoli heads were divided into florets of 10−30 g with 2 cm floret stem. 50 florets per treatment were randomly selected, steamed, cooled and vacuum-packed in boil-resistant bags, and kept at −20°C until sensory analysis. The assessors were served broccoli florets which were steamed for 6 min at 100°C in preheated porcelain bowls placed on a hot-plate. A descriptive sensory analysis was performed (ISO 6564:1985E) by a trained sensory panel of eight assessors (Nofima AS, Ås, Norway). Twenty-nine sensory attributes within flavour, taste, appearance, colour, odour, and texture were evaluated. The panelists recorded their results at individual speed on a 15 cm non-structured continuous scale. The data registration system, EyeQuestion, v. 3.8.6 (Logic 8, The Netherlands) transformed the responses from 0−15 cm on the screen to numbers from 1.0 (low intensity) to 9.0 (high intensity). Detailed information about the sensory analyses can be found in **Paper I**.

Nitrate in lettuce was determined by milling and mixing 6–10 lettuce heads from each treatment (**Paper II**). Samples of 20 g were stored at −18°C. Nitrate was extracted from the frozen samples in 100 ml boiling water and then analysed by a spectrophotometer (FIAstar 5000 analyser, Foss Analytical AB, Sweden), at NIBIO, division Apelsvoll.

2.4 Calibration and evaluation of the EU-Rotate_N model

The model calibration presented in **Paper III** was done after setting the initial pool sizes for all the organic materials. This was decided *a priori* based on literature values on the biochemical composition of the AOM pools, which is hemicellulose-/cellulose-like (AOMs) and soluble components (AOMf). The model calibration was done by inverse parameter estimation, i.e., adjusting the values for decomposition rate coefficients (*k* for AOMs and AOMf, respectively) and C:N ratio of each pool (CN_slow and CN_fast) to obtain the best possible fit between simulated and measured values of C and N mineralization from the added resources. First, decomposition rate coefficients (*k*) for pools of AOMs and AOMf of the different materials were adjusted manually until the model produced a simulation of the measured C mineralization data from the incubation experiment that gave the best possible match both visually (shape of the curve) and statistically. Next, the CN_slow and CN_fast for each organic material were adjusted to achieve the best possible fit between simulated and measured N mineralization both visually and statistically. The size, decomposition rate coefficient (*k*) and C:N ratio of each pool are listed in Table 6.

Table 6. Estimated sizes of pools of added organic matter with slow and fast decomposition (AOMs and AOMf), and calibrated values of decomposition rate coefficient *(k)* and C:N ratio for slow and fast fractions of the selected organic resources.

Organic resources	AOMs	AOMf	*k*_slow	*k*_fast	CN slow	CN fast
	(% of added materials)		(day^{-1})			
Shrimp shell pellets **(SSP)**	28	72	0.0002	0.120	2.0	6.8
Shrimp shell powder **(SSM)**	28	72	0.0001	0.200	2.5	6.1
Fish sludge waste **(FW)**	28	72	0.0005	0.130	4.0	9.3
Meat bone meal **(MBM)**	38	62	0.0001	0.100	6.0	4.4
Anaerobically digested food waste **(AD)**	72	18	0.0001	0.150	2.0	0.6
Sheep manure **(SM)**	65	25	0.004	0.080	20.0	6.4
Commercial algal meal **(AM)**	65	35	0.0001	0.005	21.0	78.4
Algal meal *Laminaria digitata* **(LD)**	65	35	0.005	0.100	13.5	62.9
Algal meal *Saccharina latissima* **(SL)**	65	35	0.0001	0.070	12.0	36.7

2.5 Model inputs for model performance evaluation

The newly calibrated model was evaluated by simulating the crop data and mineral N in soil obtained for broccoli and potato in Bodø in the years of 2009 and 2010. Information entered in the input files on management, crop species, time of planting, date of harvesting and target DM yield, is listed in Table 4 in **Paper III**.

The simulated crop growth is dependent upon the parameters critical %N and target DM yield. The target DM yield approach reduces challenges normally occurring when using photosynthetis-driven algorithms for every vegetable in the model. Each crop in the model has its own critical %N, which is the lowest crop N concentration required for maximum growth during the growth period. This is expressed in relation to the total DM yield present at any time, and is calculated as (Greenwood, 1986):

$$\text{Critical } \%\text{N} = a(1+b*e^{-0.26W}) \qquad \text{(equation 4)}$$

where W is total above-ground DM yield (Mg ha^{-1}) and a and b are crop-specific constants. Originally, a and b for broccoli were 3.45 and 0.6, respectively, and 1.35 and 3 for potato. During the model evaluation, consistent underestimation was observed for potato yield and DM for all treatments including MF. Therefore, the parameters of equation 4 for potato was adjusted to fit the yield and DM for MF potato. The a and b constants in the calibrated equation 4 were 0.70 and 2, respectively.

2.6 Statistical evaluations

2.6.1 Yield and quality evaluation

In **Papers I** and **II**, analysis of variance (ANOVA) by general linear model (GLM) was performed to determine statistically significant differences in yield, N content and quality variables between fertilizer treatments for broccoli, potato and lettuce. Fertilizer treatments were main factors (fixed), and year, location and interactions were considered as random factors. Tukey's t-test was used to determine whether differences between fertilizer treatments were statistically significant.

Linear regression was performed to test the relationship between estimated plant-available N and crop and quality data (**Paper II**). Pearson correlation analysis was performed to test relationships between glucosinolate components and plant-available N, total N, total S or N:S ratio and principal components analysis (PCA) was performed on yield and N parameters, glucosinolates and sensory attributes (**Paper I**). All statistical calculations were performed using Minitab 16, 17 and 18. A 95% confidence interval of means was used to determine whether the differences between yields, NRE, and contents of total glucosinolates and nitrate obtained after different treatments were statistically significant. The variability of the three replicates of measured mineral N in incubation experiments, is expressed as standard deviations.

2.6.2 Model calibration and evaluation of model performance

The calibration with measured C and N mineralization values and prediction of observed crop data were evaluated statistically (**Paper III)**. The latter included yield, DM, and N contents for each replicate and two years. The following statistical indices were chosen to evaluate the model calibration: mean absolute error (MAE) (Willmott, 1982), root mean squared error (RMSE) (Willmott, 1982), model efficiency (ME) (Nash and Sutcliffe, 1970), and coefficient of residual mass (CRM). MAE and RMSE include the difference between simulated and measured values, and the closer they are to zero, the better is the goodness of fit. ME compares the difference between simulated and measured values against the variance of the measured values over a period. The value ranges from –1 to +1, where –1 denotes no correlation and +1 indicates a perfect fit. If the values are negative, the simulated results are worse than using the mean of the measured data. CRM indicates the tendency to overestimate (positive values) or underestimate (negative values) the measured values. For a perfect model fit the value should be equal to zero.

$$\mathrm{MAE}=\frac{\frac{1}{n}\sum_{i=1}^{n}|P_i-O_i|}{\bar{O}_n} \quad \text{(equation 5)}$$

$$\mathrm{RMSE}=\frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i-O_i)^2}}{\bar{O}_n} \quad \text{(equation 6)}$$

$$\mathrm{ME}=1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{\sum_{i=1}^{n}(O_i-\bar{O}_n)^2} \quad \text{(equation 7)}$$

$$\mathrm{CRM}=\frac{\frac{1}{n}\sum_{i=1}^{n}(P_i-O_i)}{\bar{O}_n} \quad \text{(equation 8)}$$

where P_i is the simulated or predicted value and O_i is the measured or observed value at the i^{th} sampling instance (i = 1, 2, …, n), and $\bar{O}_n$ is the average of observed values. In the calibration experiment, O_i is the average of three replicates, whereas in the model evaluation experiment O_i represents each of three replicates. Additionally, for the field experiment, the percentage bias was calculated as:

$$\% \text{ bias}=(O_i - P_i)*100\%/O_i \qquad \text{(equation 9)}$$

3. RESULTS AND DISCUSSION

3.1 Determination of mineralization patterns of the selected organic fertilizer resources

During incubation of soil with and without organic materials (Table 1) for 60 days (*Incubation B*; **Paper III**), C mineralization rates initially ranged from relatively slow to rapid, and they converged after about 20 days towards substantially slower rates for all materials. At day 60, the resulting values of cumulative C mineralization ranged from –10 to 68% of added C (Figure 1 in **Paper III**). Net N mineralization at the end of incubation, ranged from 54 to 86% of added N for all materials except macroalgae (LD (16 %), SL (9 %) and AM (– 25 %); Figure 7). There was a significant negative relationship (R^2= 93.4%) between C:N ratio of the materials and net N mineralization (% of added N) at the end of incubation. The markedly different patterns of C and N mineralization from the organic materials fell into three groups comparable to those identified by Jensen et al (2005) in a similar, but more comprehensive study on plant residues. One group consisted of very N-rich materials of industrial origin (MBM, SSP, SSM and FW), which caused a rapid initial increase in mineral N followed by a slower increase after about 10–20 days (Figure 7). The high initial C and N mineralization rates for these materials are in accordance with results obtained in experiments with similar organic materials (e.g., Thuries et al 2001; 2002; Cayuela 2008; Pansu et al 2003, Pansu and Thuries 2003). Another group of organic materials consisted of SM and AD, with initially high values for mineral N, especially of NH_4^+-N, persistently low C mineralization rates and slow or non-detectable increase in mineral N during the incubation. Thus, these two groups contain valuable fertilizers for horticultural and agricultural crops with high N demand (Möller and Müller 2012). The third group of organic materials comprised the brown algae, which except for AM, showed initial N immobilization followed by a slow re-mineralization. Therefore, according to this experiment, the immediate N fertilizer value of seaweeds is low, however, they may be valuable as source of other nutrients, for improving soil biological activity and physical properties and increasing soil organic C in the longer term (Loveland and Webb 2003; Diacono and Montemurro 2010).

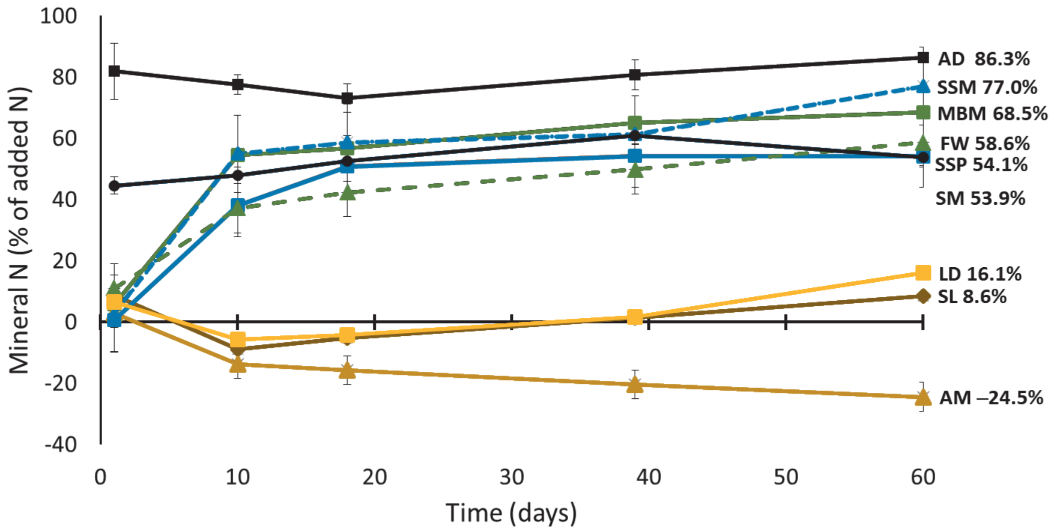

Figure 7. Net N mineralization from the waste-derived organic materials and macroalgae during 60 days of incubation at 15°C and constant moisture (*Incubation B*). Values are means of replicates (n = 3) and bars are standard deviation of the means. Abbreviation: shrimp shell pellets (SSP), shrimp shell powder (SSM), and algae meal (AM), algae meal of *Laminaria digitata* (LD), algae meal of *Saccharina latissima* (SL), fish waste (FW), meat bone meal (MBM), anaerobically digested food waste (AD) and sheep manure (SM).

In *Incubation A*, organic materials showed net N mineralization after 69 days of incubation (Figure 8). After 69 and 60 days incubation (*Incubation A* and *Incubation B*, respectively), similar N mineralization results were obtained. Also, the organic materials in *Incubation A* could be grouped into the same three groups as previously described for *Incubation B*. However, there were differences between the two experiments in net N mineralization from N-rich materials of industrial origin. The contents of mineral N were on average 14.1% and 11.5% of added N smaller for SSP and SSM, respectively, in *Incubation A* compared to *Incubation B*. For MBM the N mineralization was 11.5% of added N higher in *Incubation A*. In both experiments, the temperature was constant at 15°C and the soil was collected from the same field. Therefore, the difference might be explained by differences in soil moisture contents, as the volumetric water content was 50% versus 67% of field capacity at 5 kPa, respectively, for *Incubation A* and *Incubation B*. In addition to temperature, the main driving environmental factor for N mineralization is soil moisture. Myers et al (1982) found net N mineralization to be linearly or curvilinearly related to moisture contents ranging from –0.03 to –4.0 MPa, and the optimum moisture for net mineralization was from –0.01 to –0.03 MPa. Guntiñas et al (2012) reported optimal net N mineralization at 80% field capacity for three different soils. Therefore, considering a higher volumetric soil moisture content in *Incubation A*, higher values of net N mineralization could be expected. However, as the net N mineralization was similar in the two experiments for all materials other than SSP, SSM and MBM, it seems likely that other processes and factors than moisture might be responsible for the discrepancies.

Temperature and moisture are also important for other pathways in the N cycle after mineralization. High moisture contents and low oxygen levels increase denitrification. Typically, denitrification occurs when water-filled pore space is from 60% and higher (Robertson and Groffman 2015), which was the case for *Incubation A*. High pH increases denitrification (Bremner and Shaw 1957). The higher pH level in SSP and SSM compared to the other incubated organic materials in combination with high moisture, may therefore most likely explain the intensive nitrous oxide production from these materials in *Incubation A*, and the lower measured mineral N content. Especially for pelletized fertilizer materials there can be microsites with low oxygen level inside and around the pellets in combination with C, which gives energy for the anaerobic heterotrophic microbes responsible for denitrification (Cabrera 1994). Thus, its physical properties may explain the higher rate of nitrous gas emission from pelletized shrimp shell compared to powder, despite the similar biochemical composition of these materials. Nitrous oxide emission from the shrimp shell materials is shown in Figure 2 in **Paper IV**. Figure 1 in **Paper IV** shows a decrease in measured mineral N content for SSP simultaneously with the measured intensive nitrous gas emission for this material. These results indicate the sensitivity of environmental changes on pathways in the N cycle. The higher pH in SSM and SSP might influence the level of ammonium volatilization. High pH influence the rate of ammonium lost as ammonia, as the pH affects the ratio of NH_4^+:NH_3 (NH_4^+ reacts with OH^-). As NH_3 is a weak base, this will in turn increase pH and accelerate the loss of N as ammonia (Cameron et al 2013). Loss of N as gas (denitrification and ammonium volatilization) and nitrate is less from materials with high C:N ratio and low level of mineralized N (Robertson and Groffman 2015; Cameron et al 2013; Myers et al 1994; Swift et al 1979), e.g., algal meal. The SM and AD treatments showed high initial values of inorganic N, but additional N mineralizaion during the incubation periods was small and not detectable for SM and AD, respetively. The small differences in mineral N contents for SM and AD between the *Incubation A* and *Incubation B* are due to different initial ammonium concentrations. Even though SM and

AD were collected from the same farm and biogas production company, seasonal variation in biochemical quality is expected.

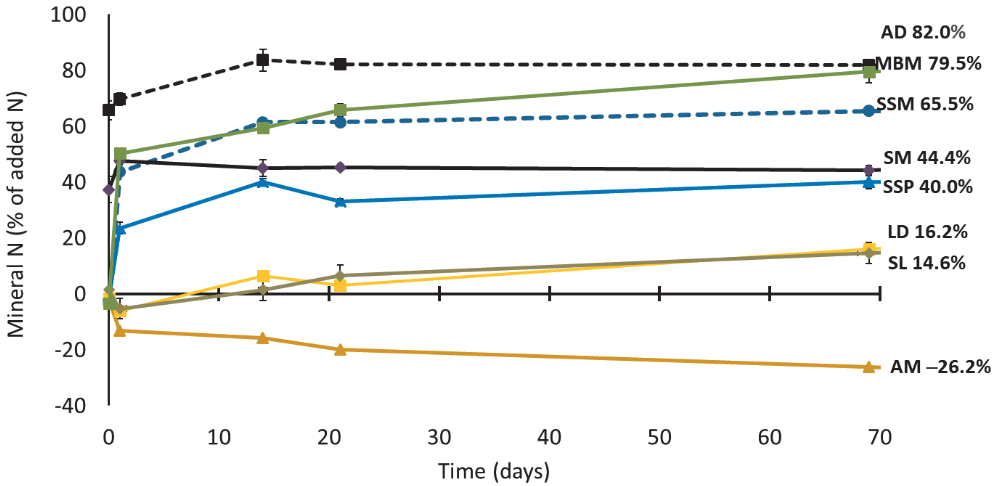

Figure 8. Net N mineralization from the waste-derived organic materials and macroalgae during 69 days of incubation at 15°C and constant moisture (*Incubation A*). Values are mean of replicates (n = 3) and bar are standard deviation of the means. Abbreviation: shrimp shell pellets (SSP), shrimp shell powder (SSM), and algae meal (AM), algae meal of *Laminaria digitata* (LD), algae meal of *Saccharina latissima* (SL), fish waste (FW), meat bone meal (MBM), anaerobically digested food waste (AD) and sheep manure (SM).

3.2 Effects of selected organic fertilizer resources on crop yield, nitrogen uptake and apparent nitrogen recovery efficiency

Selected data from **Paper II** on yield and N recovery efficiency (NRE) are presented in Figures 9 and 10. In general, there was no statistically significant difference between effects of AD, SSP and MF on yield, N uptake and NRE of broccoli, potato and lettuce fertilized with AD and SSP at the same N application rate. However, broccoli yield obtained after fertilization with 170 kg N ha^{-1} of AD and SSP were 81.5% and 75.4%, respectively, of those obtained for broccoli fertilized with MF (average for Bodø and Grimstad and the years of 2009 and 2010). Corresponding data for potato (80 kg N ha^{-1}) were 84.5% and 91.9%, and for lettuce (60 kg N ha^{-1}) 76.2% and 81.2%. Yield, N uptake and NRE values obtain with SM were lower than those obtained with AD and SSP. AM tended to give an even smaller yield than with no fertilizer

(NF) (although the difference was not statistically significant) and, hence, the NRE values with AM were negative. The effects of fertilizer type on yield, N uptake and NRE of broccoli, potato and lettuce ranged in the order of MF>AD≈SSP>SM>NF>AM. For each year and location, the yields and N uptake were positively correlated with estimated plant-available N per growing season as determined in the incubation (R^2 for N uptake varied from 48.6% to 84.8%; Figure 2 in **Paper II**). The NRE for all crops correlated with plant-available N (R^2 ranged from 35.5% to 55.6%; $P<0.001$).

The negative effect on yield and NRE found after fertilization with the seaweed product AM, was not unexpected considering its C:N ratio of 28. Immobilization has been found for materials of similar C:N ratios (Breland 1996; Jensen et al 2005; Vigil and Kissel 1991). After 60 days of incubation (*Incubation B*), net N mineralization from LD and SL, which have lower C:N ratios than AM, were small but positive (Figure 7 and **Paper III**) after an initial period of immobilization. Little is known yet about decomposition and N mineralization from seaweeds, and more research elucidating the effects of different biochemical components on mineralization of seaweeds is needed to conclude about N fertilizer effect. *Laminaria digitata* has been found to increase the contents of inorganic N in soil after application (Alobwede et al 2019).

An increase in mineral N in soil was not observed after AM fertilization in the current experiment. Addition of organic amendments with high C content to soils might improve the soil physical and biological properties (Loveland and Webb 2003; Diacono and Montemurro 2010), which is a goal of the fertilization strategy in organic farming (IFOAM 2014; European Commission 2013). The high C mineralization rates obtained during incubation of seaweeds confirms high microbial activity after application to soil. Use of seaweeds for agricultural purposes might, therefore, have other beneficial effects on agricultural crop production and soil physical quality beyond what their mineral N fertilizer replacement value would indicate. In addition, on-land use of seaweed, which has captured nutrients lost to the environment surrounding aquaculture, might contribute to recycle nutrients to terrestrial areas (Alobwede et al 2019).

A portion of 50 to about 60% of applied N supplied as MF to broccoli, potato and lettuce was recovered in crops in the current field experiments. These apparent N recovery efficiency values are in accordance with reports from other studies and under common management practice for vegetable production (Zebarth et al 1995; Congreves and Eerd 2015; Vågen et al 2005). Zebarth et al (1995) found a negative linear relationship between N fertilizer rate (125 to 625 kg N ha^{-1}) and apparent NRE in broccoli (NRE ranged from 20% to 93%). Fertilization with SSP and AD tended to result in NRE values (close to 40% for all crops) that were lower than for MF. However, the differences were not statistically significant. This tendency is as expected considering the N dynamics and N fertilizer values observed during the present incubations and considering NRE values reported for similar materials in other studies (e.g., Berry et al 2002 Möller 2015; Jeng et al 2004; Haraldsen et al2011; Brod et al 2012; Craswell and Godwin 1984; Galloway et al 2003; Raun and Johnson 1999). However, there is a potential for improving NRE by adjusting the management practice to minimize risk of losing N as nitrate or gas. This may be done by better matching of the rate and timing of plant-available N with the crop demand, and by choosing appropriate organic fertilizer materials for the crop, incorporation of organic fertilizer in soil, split fertilizer application, and by adjusting crop-related factors such as growing cultivars with deeper roots and higher plant density (Congreves and Eerd 2015; Craswell and Godwin 1984).

The utilization of such N-rich materials as fertilizers will contribute to an immediate N fertilizer effect, which makes it possible to maintain high yields also for vegetables with high N demand without using mineral fertilizers, e.g., in organic cropping systems. However, it has been discussed whether the use of such N-rich organic materials is in accordance with the organic policy and strategy (Möller 2018). Considering the low C content and the low C mineralization rates during the present *Incubation B*, which indicate low microbial activity, the use of these organic materials will to a limited extent influence soil fertility indices such as biological activity and soil organic matter. Another discussion is related to the use of waste-derived organic fertilizer of conventional origin in organic cropping systems. Anyhow, sustainable agricultural management includes nutrient use efficiency, nutrient recycling and low impact on the environment. Thus, using anaerobic digestates and N-rich organic waste materials fulfils many of these sustainability goals (Möller 2015; Möller 2018).

Challenges and concerns about soil fertility and nutrient imbalances in cropping systems with addition of organic fertilizer materials have been reviewed by Möller (2018). The main reason for these imbalances is the composition of nutrients and nutrient stoichiometry in many of these organic resources in relation to crop nutrient requirements and offtake with the harvested produce. Due to challenges of matching the plant-available N with crop demand, supplementing organic materials in order to achieve sufficient N for crops, might lead to imbalance of other essential nutrients such as P and K. As a result, the nutrient status in soils would in the long term become imbalanced. Thus, combinations of different organic fertilizer materials to meet a balanced nutrient demand for crops will contribute to a more sustainable and soil fertility-building nutrient recycling (Brod et al 2018). Möller (2018) suggested that the challenge of meeting the crop nutrient demand in organic production systems is to combine organic fertilizer with obtaining a higher share of biological N fixation.

3.3 Effects of the selected organic fertilizer on crop physical quality, sensory quality and contents of secondary plant metabolites

Broccoli, potato and lettuce fertilized with MF, AD, SSP and SM resulted in low rate of discarding due to size and physical disorders. AM-fertilized broccoli, potato and lettuce had the highest percentages of discarding due to size and physical disorders, and a high percentage not harvested due to dead plants in the field. This was expected considering the negative effect of AM on N availability (Figures 7 and 8; Doltra et al 2011). Also, the size distribution was affected by fertilizer type and application rate (Figure 1 in **Paper II**). Broccoli and lettuce fertilized with MF, AD and SSP tended to have a higher proportion of larger broccoli heads (>100 mm) and lettuce heads (>350 g). For potato, the highest proportion of large tubers was obtained with AM (**Paper II**).

Nitrate in lettuce and glucosinolates in broccoli were influenced by fertilizer type and application rate. In lettuce, the highest concentration of nitrate (mean of three replicates: 157.3 mg kg^{-1} fresh weight) was obtained after MF-fertilization at Bodø location (**Paper II**). There was no statistically significant difference between the organic fertilizers for nitrate concentration of lettuce (Figure 11). Due to low N fertilization rates (60 kg N ha^{-1}) the nitrate concentrations in crops were low compared to results in other studies (e.g., Santamaria 1999; 2006) and low compared to the acceptable daily intake of nitrate, which is <222 mg day^{-1} for a 60 kg human (EFSA 2008).

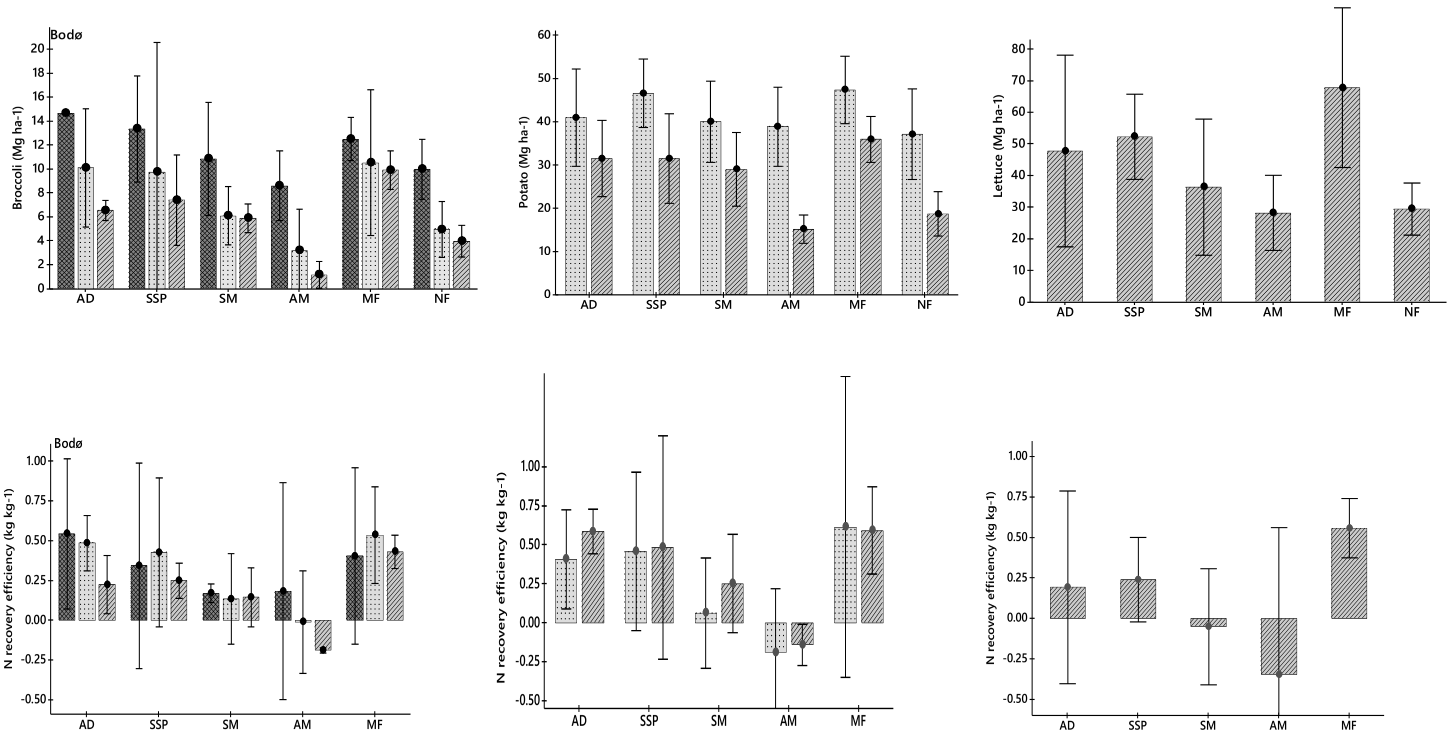

Figure 9. Yield and N recovery efficiency of broccoli, potato and lettuce grown with and without (NF) fertilizer. Broccoli, potato and lettuce were fertilized with anaerobically digested food wastes (AD), shrimp shell pellets (SSP), sheep manure (SM), algal meal (AM) and mineral fertilizers (MF) at 170, 80 and 60 kg N ha^{-1}, respectively. The experiment was conducted at Bodø for three (2008, 2009 and 2010), two (2009 and 2010) and one (2010) year(s) for broccoli, potato and lettuce, respectively. Bars are 95% confidence intervals of means.

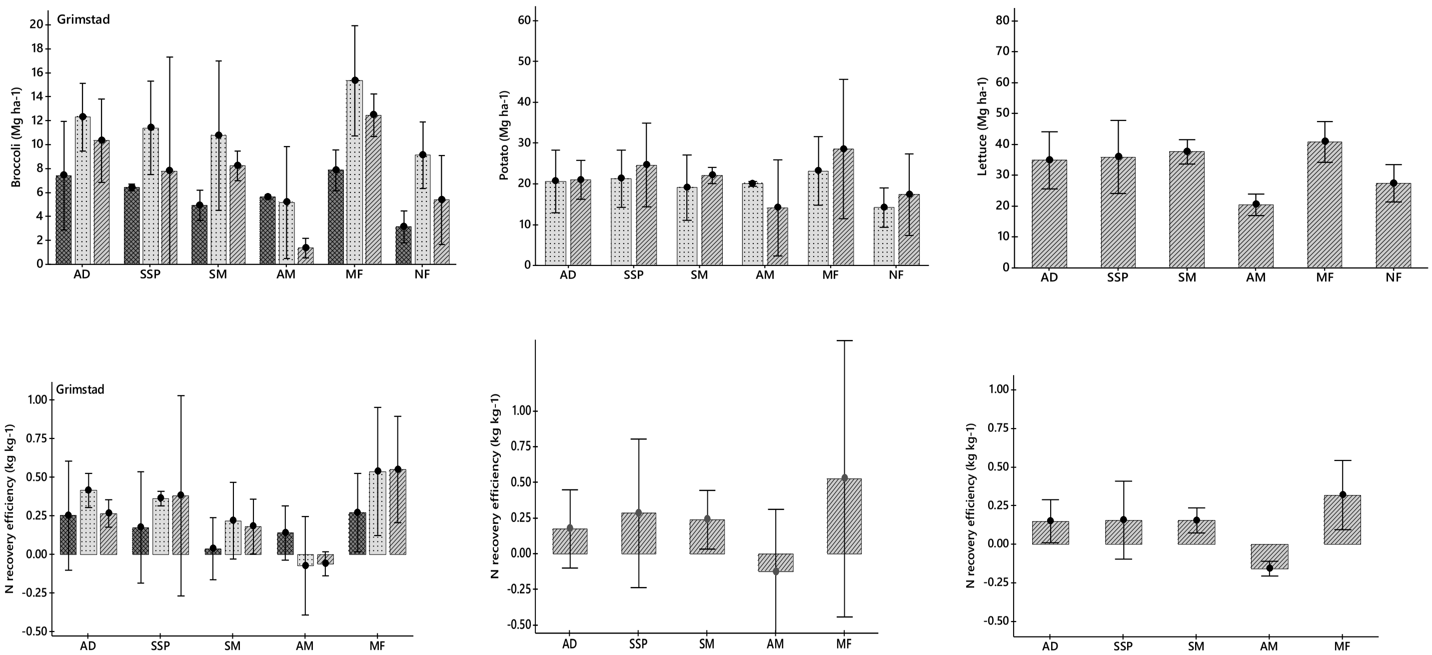

Figure 10. Yield and N recovery efficiency[2] of broccoli, potato and lettuce grown with and without (NF) fertilizer. Broccoli, potato and lettuce were fertilized with anaerobically digested food wastes (AD), shrimp shell pellets (SSP), sheep manure (SM), algal meal (AM) and mineral fertilizers (MF) at 170, 80 and 60 kg N ha^{-1}, respectively. The experiment was conducted at Grimstad for three (2008, 2009 and 2010), two (2009 and 2010) and one (2010) year(s) for broccoli, potato and lettuce, respectively. Bars are 95% confidence intervals of means.

[2] NRE for potato 2009 was calculated due to lack of residues harvesting.

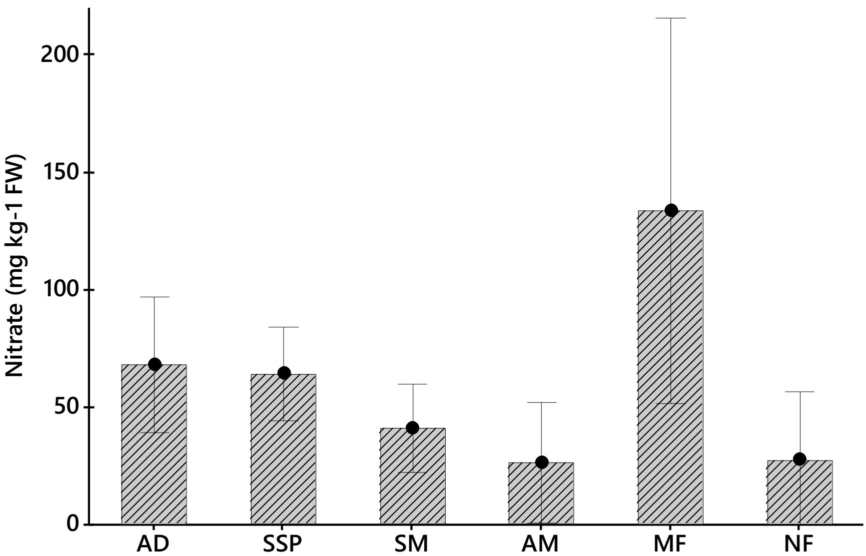

Figure 11. Average nitrate concentration (mg kg^{-1} fresh weight) in lettuce grown in Bodø and Grimstad in 2010. Interval bars are 95% confidence intervals of means.

The results from the analysis of total glucosinolate content indicate that broccoli fertilized with SSP differ from the other treatments (Figure 12). The differences of SSP- and MF-fertilized broccoli were not statistically significant (**Paper I**). Total glucosinolate, total indolic and total aliphatic glucosinolate contents were found to be highest for broccoli fertilized with MF and SSP and lowest for SM and NF. The content of total glucosinolate ranked in the order SSP>MF>NF>SM. The contents did not correlate with total N or estimated plant-available N, although S content correlated with the glucosinolate contents. This is in accordance with results found by Li et al (2007) and Kestwal et al (2011). The total indolic glucosinolate and glucobrassisin correlated with total N, estimated potentially plant-available N and total S content in the organic fertilizers, as found by Kim et al (2002). Thus, the higher content of glucosinolates in SSP and MF cannot be explained solely by N fertilization or availability but must be seen in relation to S status. Another explanation is that SSP, which is high in chitin, might induce a stress response that can influence the biosynthesis of secondary plant metabolites such as glucosinolates (Bautista-Baños et al 2006; Bourn and Prescott 2002; Young et al 2005).

A significant effect of fertiliser type and application rate was observed for 16 of 29 sensory attributes evaluated for broccoli (**Paper I**). The differences in score for individual attribute was small and ranged from 2.2 to 12.2%. There was no obvious trend in how the organic fertiliser materials and their N contents or estimated amounts of potentially plant-available N influenced the sensory quality. Part of the differences may be explained as an indirect effect of applied fertilizers due to crop maturity at harvest, which has been found to influence sensory attributes (Talavera-Bianchi et al 2010).

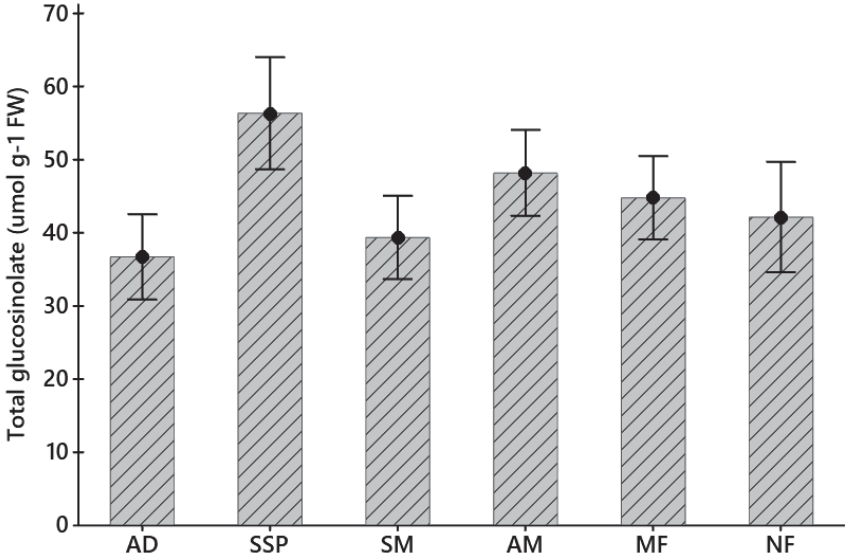

Figure 12. Total glucosinolate contents in sinigrin equivalents (µmol g^{-1} fresh weight) in broccoli florets after AD, SM, SSP, AM and MF at 170 kg N ha^{-1} rates, and non-fertilized florets (NF). Results are means of three years (2008, 2009 and 2010) and two locations (Bodø and Grimstad). Interval bars are 95% confidence interval of means.

3.4 Calibration of the EU-Rotate_N model

With some exceptions, initialization and calibration of the N mineralization module of EU-Rotate_N (i.e. inverse estimation of the values of the decay rate constantes and C:N ratios of the AOM pools), produced reasonably good fits with the observed C and N mineralization in

Incubation B (Table 5 and Figure 4 in **Paper III**). For N-rich organic resources originating from industry (MBM, SSP, SSM and FW), the calibration was successful. However, for SSP there was poor correlation (low ME values) between measured and simulated C mineralization (Table 5 and Figure 4 in **Paper III**). This poor correlation could be due to the model's inability to take explicit account of effects of physical property of the organic material. Despite being similar in chemical composition, C and N mineralization differed between SSP and SSM. These differences can most likely be explained by the physical properties of the pellets, which may retard microbial colonization and decomposition, partly through locally intense oxygen consumption, which might also favour N dissimilation by denitrification (Cabrera et al 1994). This interpretation is supported by a higher nitrous oxide emission rate measured for SSP than for SSM in *Incubation A* (**Paper IV**). During the time prior to the measured intensive nitrous oxide production from SSP, a decrease in mineral N was observed, approximately equivalent to the amount of N in the observed nitrous oxide emission (Figure 1 and 2 in **Paper IV**). With the previously discussed exceptions for SSP and MBM, the measured values of mineral N (kg ha^{-1}) from the incubated N-rich organic fertilizer materials *(Incubation B*) used to calibrate the EU-Rotate_N model, corresponded to measured mineral N in the independently performed incubation experiment (*Incubation A*) (Figure 13 and Table 7). *Incubation A* thus validates the N mineralization data obtained in *Incubation B* which were used for calibration.

For some of the other materials (seaweed, AD and SM) it was difficult to match equally well the measured C and N mineralization obtained during incubation by adjusting the decay rate constants and C:N ratios. The partitioning of C between AOMs and AOMf for AD was set at the model's default values for animal manures and slurries, while for SM a somewhat larger AOMf fraction was chosen because of its content of straw. The relatively good fit between simulated and estimated C mineralization for SM suggests that this was a correct decision, however, the correlation indices for N mineralization were poor for results obtained in both incubation experiments (Figure 13 and Table 7). For AD, the opposite was the case, with poor fit with C data and good fit with N data.

The partitioning of C to the fast pool AOMf, guided by the amounts of structural compounds in brown algae as taken from the literature (Øverland et al 2017; Schiener et al 2015), seems to be adequate for SL and LD, but not for AM. The decay rate constants for AOMf estimated by calibration ranged from 0.005 to 0.100, lowest for AM and highest for LD. Simulated N mineralization from LD and SL visually showed very good fits with measured values obtained in *Incubations A* and *Incubation B* (Figure 13), but simulated values for AM were less negative than measured values. The low *k* values for AM are atypical, which can be explained by biochemical properties not accounted for, but N-limitation may also be a factor, as very low concentrations of inorganic N were measured in soil with AM. Reduction of C mineralization under decompositions of structural materials has been found under restricted N conditions (Henriksen and Breland 1999). The EU-Rotate_N model has a routine for taking account of N-restricted decomposition, but it may not be restrictive enough for the conditions in the present experiment. In addition, differences in C and N mineralization between AM, SL and LD were likely due to species-specific differences in chemical composition (Schiener et al 2015), e.g., the contents of polysaccharides (laminarin, mannitol, alginate, fucoidan, cellulose), monosaccharides, polyphenols, protein, ash, and total C and N. Of these, the contents of laminarin and polyphenol are higher in SL compared to LD, and alginate contents are lower in SL (Schiener et al 2015). Studies of animal digestion of brown algae suggest that a high content of polysaccharides renders the material more recalcitrant, especially in combination with phenolic compounds (Øverland et al 2017). This might explain the lower decay constant for SL compared to LD, despite lower C:N ratio for SL. Values of N mineralization in *Incubation A* validated the N mineralization data used to calibrate the model for AD, SM and seaweeds.

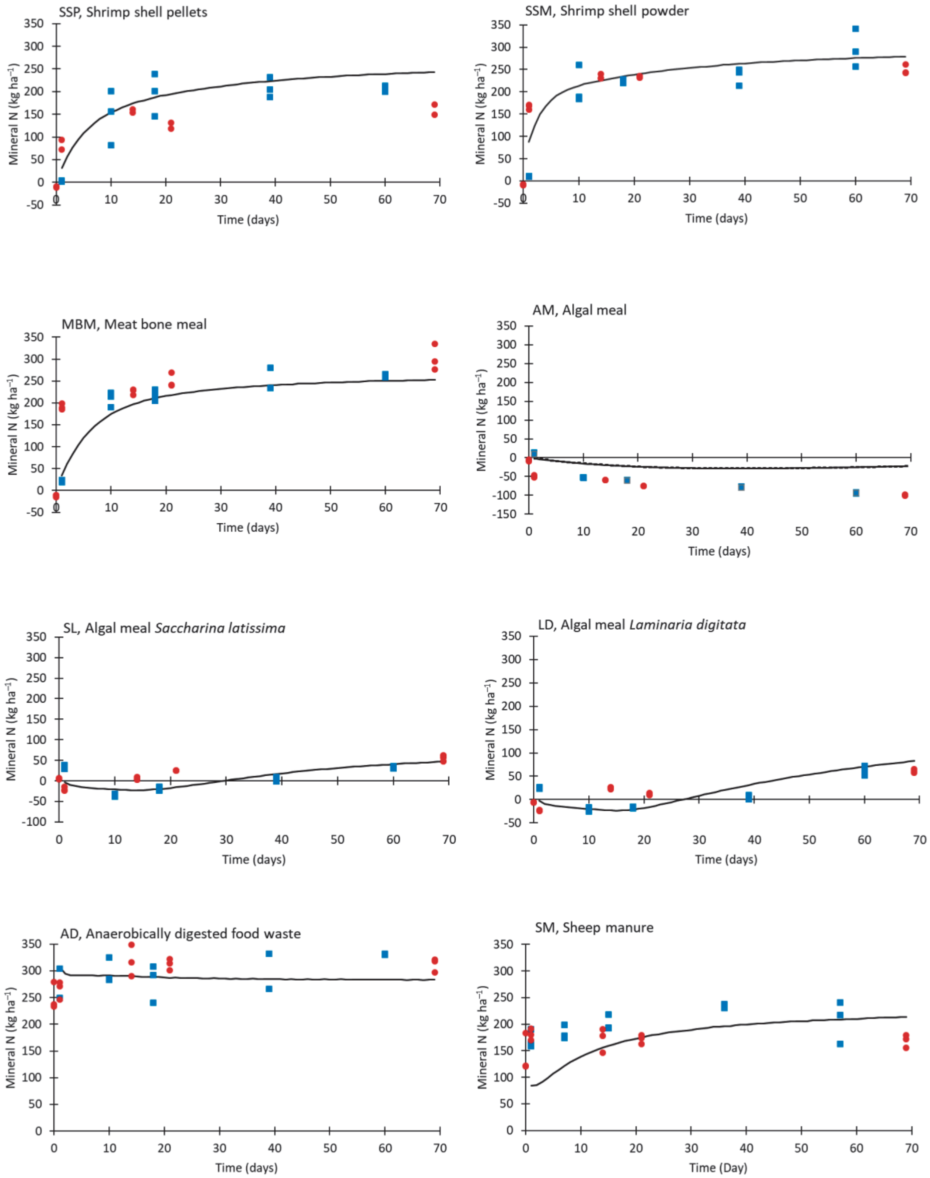

Figure 13. Measured values of mineral N (kg ha^{-1}) from organic resources incubated at constant temperature and soil moisture (*Incubation B*; blue squares), values (lines) simulated by calibration of the EU-Rotate_N model calibrated with C and N mineralization data from *Incubation B* and measured mineral N in an independently performed incubation experiment (*Incubation A*; red dots).

Table 7. Statistical parameters for goodness of fit between simulated and measured values of N mineralization (kg ha^{-1}) from eight incubated organic resources as obtained by calibrating EU-Rotate_N (incubation B) and by applying the calibrated model to predict N mineralization in the independently performed incubation A. For explanation of the abbreviations of the organic resources, see Tables 1 and 2.

	MAE		**SRMSE**		**ME**		**CRM**	
Incubation nr	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
SSP	0.58	0.12	0.63	0.14	0.06	0.93	0.30	0.10
SSM	0.18	0.14	0.24	0.20	0.85	0.85	–0.07	0.10
MBM	0.35	0.08	0.44	0.09	0.50	0.96	–0.35	–0.05
AM	**–0.92**	**–0.75**	**–0.95**	**–0.82**	**–1.64**	**–0.56**	–0.92	–0.66
SL	**1.64**	**4.04**	**1.84**	**5.37**	0.12	0.53	–1.06	–0.21
LD	**2.15**	**1.22**	**2.30**	**1.54**	0.05	0.69	–0.59	0.07
AD	0.11	0.13	0.11	0.23	**–0.01**	**–0.53**	–0.03	–0.12
SM	0.24	0.23	0.32	0.27	**–12.37**	**–5.51**	–0.10	–0.23

3.5 Evaluation of the model's ability to predict crop and soil data from the field trial conducted in Bodø

Predicted and mean observed values for broccoli and potato yields, DM of yield (DM_{yield}) and total plant material (DM_{total}), N in the entire plant (N_{total}), and soil mineral N (N_{soil}) are presented in Table 6 in **Paper III**. The statistical indices describing goodness of fit can be found in Table 7 in **Paper III**. The EU-Rotate_N model predicted the observed values for DM and N uptake quite well for broccoli after fertilization with MF, AD, SSP and SM using the original default values for critical %N for optimal crop growth. However, the potato yield and the other crop data could not be predicted with the model's default values for critical %N, as the model underestimated these values for all fertilizer treatments. The adjustment of critical %N for potato improved the model's ability to simulate the potato crop variables. Soil N variables and variables obtained by AM-fertilized potato and broccoli were more poorly predicted. The poor correlation for AM in the evaluation experiment was in line with the poor fit between simulated and measured C and N mineralization under controlled temperature and moisture conditions. For unfertilized (NF) broccoli, there was a substantial lack of fit, but the predictions of observed potato values were satisfactory. In addition to critical %N, the DM target yield input in the model was crucial for the accuracy of the model prediction. Thus, the sensitivity of the model to values of input variables illustrates that it must be used with caution, maybe in combination with other models, as a decision support tool (Palosuo et al 2010; Rötter et al 2012).

The deviations between predicted and observed values for AD, SSP and MF are within the range of other statistical evaluations of the model (Nendel et al 2013; Rahn et al 2010; Soto et al 2018). Nendel et al (2013) similarly found that the model satisfactorily predicted DM and N contents of crops, but that soil mineral N predictions were poor. The underestimation of soil mineral N in the present study is in accordance with other studies (Soto et al 2018; Doltra and Muñoz 2016). The underestimation might be explained by either underestimation of N mineralization or an excessively high critical %N curve. In the model, both will contribute to N-limited crop growth. In the case of AM, overestimation of N mineralization was certainly the major explanation for the poor fit between predicted and measured values.

4. CONCLUSIONS

4.1 Key findings

Results from the incubation experiements showed that the N mineralization potential of organic resources relevant as fertilizers ranges from negative to substantial (RQ1) and support the assumption that the C and N mineralization patterns differ widely as a function of biochemical composition of the materials (H1). Accumulated N mineralization was linearly related to C:N ratio of the selected organic materials. For materials with high content of inorganic N at application or high net N mineralization during the growing season (shrimp shell pellets, shrimp shell powder, meat bone meal, fish waste sludge and anaerobically digested food waste), 54% to 86% of the added N was recovered as mineral N during incubation. Such organic fertilizer resources thus seem to have a potential for replacing or supplementing mineral fertiliser in conventional production systems and to be a complementary fertilizer resource in organic production. The N mineralization from the seaweeds *Laminaria digitata* and *Saccharina latissima* was moderate during incubation (16% and 8% of added N, respectively), and even negative for the commercial algal meal (–25% of added N).

For materials with high N mineralization potential, the N fertilizer effect measured as yield response and N use efficiency of vegetable crops in the field trials was similar to that of inorganic N fertilizer, whereas other materials performed substantially poorer (RQ2). Yield and N recovery efficiency could be explained by potential N mineralization during the growing season as estimated from data from the incubation trials (H2). Incubation studies for determining N mineralization patterns of organic fertilizer resources seem to be a useful, albeit time-consuming, tool for selecting the type, rate and timing of organic fertilization, towards best management practice for optimising economic returns and reducing negative environmental impacts.

The tested organic fertilizers influenced vegetable quality (RQ3). External product quality parameters such as size and physical disorders were correlated with the estimated net N mineralization. Also, the concentration of nitrate in lettuce was affected by the type of fertilizer. However, the effects on sensory quality and contents of biochemical compounds in vegetables was less clear (H3); differences in sensory attributes were related more to year than to fertilizer material and location. Glucosinolate contents were influenced by the type of organic fertilizer. However, there was no correlation with the measured net N mineralization. Total and individual glucosinolate contents must be seen in relation to factors other than N, such as sulphur. The high content of glucosinolates in broccoli fertilized with shrimp shell powder might be explained either by this material's contents of N and S or by its C:N ratio, or else by the plants' natural defence metabolites activated as a result of the presence of chitin.

The assumption that the EU-Rotate_N model can describe C and N mineralization dynamics of selected organic materials under controlled temperature and moisture conditions (H4a) was in part supported, but some challenges regarding calibration with C and N mineralization data from seaweed suggests a need for more information about decomposition of these materials. For the brown algae *Laminaria digitata* and *Saccharina latissima*, model calibration with C and N mineralization data produced visually good fits with measured data, but poorer ones for algal meal. Therefore, more knowledge about brown algae decomposition is needed, including effects of N limitation, before the model can be used as a decision tool for fertilization with seaweed. Shrimp shell pellets was also challenging to calibrate. The physical properties of shrimp shell pellets compared to powder influences the emission of nitrous oxide, thus, the EU-Rotate_N model should be further improved to include physical properties on N availability (N mineralization and denitrification), in addition to the chemical composition of the organic fertilizer materials. For the fertilizer materials except seaweed, the model predicted yield and other crop data in the field trial quite well, but soil N was difficult to predict (RQ4 and H4b). The poor predictions for seaweed was not surprising considering that this group was represented in the field trial by algal meal, for which calibration with C and N mineralization data was poor.

4.2 Further investigations

Performing incubation trials to determine the mineralization pattern of N from organic fertilizer materials is an important tool for estimating their fertilizer potential. However, estimation of N fertilizer value based on measured N mineralization from N-rich organic materials, should preferably be complemented by measurements of gaseous N emissions measurements (denitrification and ammonia volatilization). The results of the calibration of the EU-Rotate N model with N mineralization data in this experiment indicates that the module determining the prediction of gaseous loss of N needs to be improved.

Mineralization of seaweed N and its fertilizer value are not fully understood. Decomposition and biochemical properties of seaweed (e.g., carbohydrates as polyphenol, alginate, fucoidan and laminarans) differ. Therefore, further investigations and knowledge about N mineralization, immobilization and re-mineralization processes are needed to determine fertilizer value and best management practices use of seaweeds in for agriculture. This knowledge is required before using the EU-Rotate_N model as a decision tool regarding seaweed as fertilizers.

Still unresolved challenges that reduce the model's value as a decision support tool are the need for setting a target yield and the supposedly variable values of critical %N among different crops and possible growing conditions. As a decision tool for fertilizer management for optimum yield, economic outcome and environmental impact, EU-Rotate_N should preferably be used in combination with other models.

Further investigations are needed to conclude about how the use of chitin-containing organic fertilizer materials impacts on product quality and contents of health-related components.

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Paper I

Øvsthus I, Breland TA, Hagen SF, Brandt K, Wold AB, Bengtsson GB and Seljåsen R, 2015. Effects of organic and waste-derived fertilizers on yield, nitrogen and glucosinolate contents, and sensory quality of broccoli (*Brassica oleracea* L. var. *italica*). Journal of Agricultural and Food Chemistry 63:10757−10767

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effects of Organic and Waste-Derived Fertilizers on Yield, Nitrogen and Glucosinolate Contents, and Sensory Quality of Broccoli (*Brassica oleracea* L. var. *italica*)

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ABSTRACT: Organic vegetable production attempts to pursue multiple goals concerning influence on environment, production resources, and human health. In areas with limited availability of animal manure, there is a need for considering various off-farm nutrient resources for such production. Different organic and waste-derived fertilizer materials were used for broccoli production at two latitudes (58° and 67°) in Norway during two years. The fertilizer materials were applied at two rates of total N (80 and 170 kg ha^{-1}) and compared with mineral fertilizer (170 kg ha^{-1}) and no fertilizer. Broccoli yield was strongly influenced by fertilizer materials (algae meal < unfertilized control < sheep manure < extruded shrimp shell < anaerobically digested food waste < mineral fertilizer). Yield, but not glucosinolate content, was linearly correlated with estimated potentially plant-available N. However, extruded shrimp shell and mineral NPK fertilizer gave higher glucosinolate contents than sheep manure and no fertilizer. Sensory attributes were less affected by fertilizer material and plant-available N.

KEYWORDS: *glucosinolates, sustainability, Brassica oleracea, broccoli, sensory attributes, nitrogen mineralization, yield, organic farming, organic fertilizer*

■ INTRODUCTION

Organic agricultural production is increasing in Europe.[1] Important reasons are consumers' growing interest in food safety, environmental impact, and sustainability of production systems as well as a preconceived notion about a superior quality of organic products with respect to nutrients, compounds with health-promoting properties, and taste characteristics.[2−6] Ethical concerns and decrease in consumers' trust in food quality also seem to be among the driving forces.[7,8] Still, price as influenced by efficiency in the production and distribution chain, including marketable crop yield per unit area, is an important determinant of consumers' choice.[9]

The ban on mineral fertilizers is one of the key characteristics of organic cropping systems. This particularly influences nitrogen (N) availability, which is the single factor that most often limits crop yield.[10] N availability during the growth of vegetables also influences several quality parameters through nitrogen's functions as building blocks in plant tissues and in metabolic and physiological processes, including synthesis of vitamins and secondary metabolites. Overall, as compared to conventional produce, organic vegetables and fruits tend to have higher contents of defense-related secondary metabolites, which comprise many of the known and supposedly health-promoting compounds in these foods.[11] Previous studies suggest that this difference may be related to N availability in cropping systems.[12]

Organic farming systems mainly depend on N_2 fixation in leguminous plants and green manure crops. On stockless farms in areas with few animals, the possibilities are limited locally for utilizing the legumes needed in crop rotations and for recycling nutrients as animal manure. The resulting high cost of N on such farms, therefore, tends to limit the proportion of legumes in the crop rotation. Hence, there is a need to consider various off-farm N sources derived from organic materials,[13] particularly for farms producing organic crops with large N demand, such as cruciferous vegetables. Organic waste materials originating from food or seafood production are potentially relevant nutrient sources. Turning such wastes into a production resource by establishing closed nutrient cycles would contribute to sustainable management of both the environment and production.

The N fertilizer effect of such resources on crop growth depends on the amount and timing of inorganic N availability in relation to crop demand.[14] The N supply from a specific fertilization source can be described as a function of the amount of total N applied, the percentage of inorganic N at application, the decomposition rate of the organic fraction, and the carbon-to-nitrogen (C:N) ratio of the fractions available to

Received: September 22, 2015
Revised: November 5, 2015
Accepted: November 9, 2015
Published: November 9, 2015

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

Table 1. Planting Date, Number of Growing Degree Days (GDDs), Growth Days (GDs), and Mean Day Temperature, Total Precipitation, and Total Sunshine Hours per Growing Season and Month in Bodø and Grimstad for the Years 2009 and 2010

					mean day temperature (°C)				total precipitation (mm)				total sunshine (h)			
location	year	date	GDDs	GDs	growing season	June	July	Aug	growing season	June	July	Aug	growing season	June	July	Aug
Bodø	2009	June 10	823	60	13.7	10.5	14.3	14.4	74.7	51.3	30.5	106.7	507.8	255.7	200.5	141.9
	2010	June 9	697	58	11.9	8.7	13.3	12.4	182.2	91.4	110.3	50.9	274.0	184.8	160.6	152.1
Grimstad	2009	May 29	979	62	15.8	14.9	16.8	15.9	296.4	52.7	243.7	98.6	578.1	276.3	198.7	157.1
	2010	June 4	1116	68	16.2	15.1	17.0	16.0	198.6	30.1	67.9	130.7	583.8	278.2	199.6	177.4

Table 2. Chemical Properties and Texture of the Upper 0.3 m Soil Layer of the Experimental Fields in Bodø and Grimstad, 2008

	chemical properties						texture		
location	pH	TC[a] (g kg^{-1})	TN[b] (g kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	P (mg kg^{-1})	sand	silt	clay
Bodø	6.1	21	1.7	7.0	3.9	840	38	52	4
Grimstad	5.9	30	1.6	11.1	1.2	790	87	10	3

[a]TC, total carbon. [b]TN, total nitrogen.

decomposers. The crop N demand typically follows a sigmoidal pattern and is defined as the N uptake over a period that allows the maximum production of dry matter.[15] An important indicator of N demand is the critical plant N concentration (PNC_c), which is the lowest level of N allowing optimum growth.[14,16,17]

Cruciferous vegetables are important dietary sources of several minerals, vitamins, and other health-related components.[6,18,19] Especially broccoli (*Brassica oleracea* L. var. *italica*) is considered an important commercial and dietary vegetable, representing a good source of glucosinolates (GLS), phenolic compounds, vitamin C, and carotenoids.[6,20,21] Consumption of cruciferous vegetables is associated with a reduced risk of certain types of cancer and cardiovascular diseases,[18−21] and this has been related to its content of GLS and their degradation products. There is also a general belief among consumers that broccoli is a healthy food.[9]

Despite this focus on chemical composition, little is known about the effects of different fertilizers on sensory quality and the content of GLS. Staley[22] found a higher content of GLS in cabbage fertilized with chicken manure and green manure compared to mineral fertilizer. In a study based on commercial broccoli purchased at monthly intervals during one year, higher levels of glucobrassicin were found in organic broccoli compared to conventional.[23] In addition to N availability, other qualities such as supply of sulfur (S)[24] and nitrogen-to-sulfur (N:S) ratio[25] as well as chitin[26] may influence GLS biosynthesis. Sensory attributes of vegetables grown organically and conventionally show inconsistent results as well,[27,28] and no assessment concerning taste of broccoli related to fertilizer materials is known.

The aim of the present study was to investigate effects of potential organic fertilizers on yield, N and GLS contents, and sensory attributes of broccoli grown at two latitudes with different climates. Algae meal (AM), extruded shrimp shell (SS), sheep manure (SM), and anaerobically digested food waste (AD) were applied at two levels of total N (80 and 170 kg ha^{-1}) and compared with no fertilizer (NF) and mineral fertilizer (MF). Particular attention was paid to possible relationships between estimated N mineralization potential of the fertilizers and parameters of yield and crop quality.

■ MATERIALS AND METHODS

Site Description, Soil Properties, and Weather Data. The experimental fields were located at the Norwegian Institute for Agricultural and Environmental Research, Division Bodø (northern Norway, 67°28′ N, 14°45′ E) and Division Grimstad (southern Norway, 58°34′ N, 8°52′ E) during the growing seasons of 2009 and 2010. The field in Bodø had been organically managed as cattle pasture for more than 25 years, whereas the field in Grimstad had been used for organic grass seed production (*Phleum pratense* L.) for 3 years. The field in Bodø was a sandy orthic humo-ferric podzol,[29] whereas the field in Grimstad was a gleyed sombric brunisol[30] with southwest-facing slopes of 2−4 and 2−6%, respectively.

The year prior to the experiments, fields were plowed (20−30 cm depth) in late July and harrowed (5−10 cm depth) twice (early August and late September) to reduce weeds. Ryegrass (*Lolium multiflorum* var. Westerwoldicum) was sown in plots prior to the experimental years. Soil samples (0−30 cm depth) were randomly taken from each replicate at both locations with a soil auger (6−10 soil cores per sample) in spring. Meteorological data during the experimental period were available on a hourly basis from climate stations near the research sites (Table 1).

Design and Management of the Field Experiments. Seeds of broccoli (*B. oleracea* L. var. *italica* cv. Marathon) were sown in plugtrays with 63 mL $plant^{-1}$ of organic peat-based compost (Norsk økotorv, Norgro AS, Ridaby, Norway) supplemented with 3 g L^{-1} of organic chicken manure (Marihøne, Norsk naturgjødsel AS, Voll, Norway). A multifactorial field experiment, with the fertilizer materials as independent variables, was established as part of a yearly crop rotation (broccoli, potatoes, lettuce). Fertilizer materials were algae meal (AM) (Bioalg regular, Nordtang AS, Vestbygd Norway), extruded shrimp shell (SS) ("Rekeskall Ottar", Produsentorganizasjonen Ottar, Finnsnes, Norway), sheep manure (SM) (Noncommercial product, Organic farm, Tjøtta, Norway), and anaerobically digested food waste (AD) (Biotek AS, Porsgrunn, Norway) supplied at two levels of N (80 and 170 kg N ha^{-1}), broadcast by hand, and incorporated to the soil by a rotary harrow. No fertilizer (NF) and 170 kg N ha^{-1} of mineral fertilizer (MF) given by a combination of NPK 12−4−18 and calcium nitrate fertilizers (Kalksalpeter) (59% of N from NPK), both obtained from Yara (Oslo, Norway), were used as control plots. The first year the total amount of organic fertilizers and 50% of the MF were added before planting, whereas the remaining MF was top-dressed twice (25% after 4 and 25% after 6 weeks). The second year, all fertilizers, except AM, were applied the same way as MF (the change was based on first-year results, which suggested nutrient runoff). Due to the low level of potassium (K) in SS, potassium sulfate (Kaliumsulfat, Kali, Felleskjøpet, Norway) was

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

supplied in SS plots in a level corresponding to the K level given by the other fertilizer materials (fertilizer rate equal to a N:K ratio 1:1). The experimental fields were arranged as a randomized block design with three large plots (30 m × 5.6 and 30 m × 6.4 m in Bodø and Grimstad, respectively), each of which was divided into 10 subplots (6 × 2.8 m and 6 × 3.2 m in Bodø and Grimstad, respectively). Six-week-old seedlings were transplanted the first week of June in rows of 18 plants and four rows per fertilizer plot. The distance between plants in the row was 33 cm, and the distance between rows was 70 and 80 cm in Bodø and Grimstad, respectively. The experimental fields were covered by floating row cover as insect net (Novagryl floating row cover, 22 g m^{-1}, pr. no. 255094, Vekstmiljø AS, Sandnes, Norway).

Nutritional Status of Soil and Organic Fertilizers. The soil samples and organic fertilizers were analyzed by Eurofins (Eurofins Food & Agro Testing Norway AS, Moss, Norway). Samples of soil and organic fertilizer materials were dried at 40 °C, strained through a 2 mm sieve, and ground in a mortar before analysis. Total carbon (TC) in soil samples for Grimstad and total N (TN) in soil samples from both locations were determined according to AJ31, a modified version of NS-EN 13137:2001. TC data for Bodø present in Table 2 were analyzed by Haraldsen et al.[29] For the organic fertilizer materials, total organic carbon (TOC) was determined according to NS-EN 1484 and AJ31, whereas total Kjeldahl N (TKN) was analyzed according to NS-EN 13654-1 and Tecator ASN 3503/300.

NO_3^--N and NH_4^+-N were extracted using 2 M KCl, whereas for the determination of phosphorus (P), potassium (K), and sulfur (S), samples were digested in 7 M HNO_3. NO_3^--N, NH_4^+-N, P, K, and S were determined according to NS-EN ISO 11885. Soil properties for the field locations and nutritional status of the organic fertilizers are given in Tables 2 and 3, respectively.

Sampling and Sample Preparation. Broccoli heads were harvested at maturity of individual plants as defined by developmental stage of flower buds (closed bud diameter of 1−1.5 mm, before elongation of bud stem) and by the density of heads (shift from compact and hard to slightly softer when the top of the head is pinched by a finger). Broccoli heads that failed to reach normal and uniform bud maturity were harvested when primary buds in the florets started stem elongation and extended 2−3 mm above undeveloped flower buds (some single buds fulfilled development). The weight of individual broccoli heads was measured, and total yield was calculated as the weight of all broccoli heads harvested in plots divided by harvested area (14.8 and 16.9 m^2 for Bodø and Grimstad, respectively). Total number of harvested broccoli heads per plant and fraction of small heads (diameter < 6 cm) were also recorded (according to NS 2823:1999).

For sensory and chemical analyses, 10 broccoli heads were divided into florets of 10−30 g with 2 cm floret stem, and 50 florets per treatment were randomly selected. For chemical analyses, florets equivalent to 200−300 g were frozen in liquid N, crushed in a mortar, and stored at −80 °C until analysis. For sensory analyses, 26 florets were steamed in a steam oven (HBC 26D550702, no. 100185, Bosh GmbH, München, Germany) until the core temperature of the broccoli floret stems was 90 °C and then steamed for an additional minute. The florets were cooled at room temperature for about 3 min and then single frozen in aluminum trays at −20 °C. The florets were vacuum packed in boiling-resistant vacuum bags (Goffrato, Scheie & Co., Bergen, Norway) in a single layer and kept in the dark at −20 °C until sensory analysis.

Nitrogen and Dry Matter Content of Plants. Total N and dry matter (DM) contents of plants were determined by harvesting (cut at soil level) 6−10 broccoli plants at maturity from each plot. The plants were divided into edible parts (broccoli heads) and nonedible parts (leaves and stem). The broccoli fractions were cut in pieces (approximately 1−2 cm in diameter and length) and mixed. Subsamples of about 500 g were dried at 60 °C for determination of DM and subsequent analysis of total N according to the Kjeldahl method.[31]

Estimation of Potentially Plant-Available N. Fertilizer-derived N potentially available to plants during the growing season was estimated using data for N mineralization obtained by incubation

Table 3. Chemical and Physical Properties of the Organic Fertilizers: Anaerobically Digested Food Waste (AD), Shrimp Shell (SS), Sheep Manure (SM), and Algae Meal (AM)

	chemical properties												physical properties
fertilizer	pH	DM %	TOC[a] (g kg^{-1} DM)	TKN[b] (g kg^{-1} DM)	NH_4^+-N (g kg^{-1} DM)	NO_3^--N (g kg^{-1} DM)	C:N ratio	EPAN (%)[c]	P (g kg^{-1} DM)	K (g kg^{-1} DM)	S (g kg^{-1} DM)	N:S ratio	
AD	8.6	1.3	307	254	153	0	1.2	86.3	18	106	8	38.4	liquid part
SS	9.2	90.2	301	72	0	0	4.2	54.1	27	1	4	2.2	dried and pelleted
SM	8.8	19.4	396	37	13	0	17.4	53.9	9	22	5	6.1	solid part, containing traces of straw
AM	6.0	89.1	406	11	0	0	36.9	−24.5	1	16	26	0.4	dried and crushed seaweed, mainly *Ascophyllum nodolus*

[a]TOC, total organic carbon. [b]TKN, total Kjeldahl nitrogen. [c]EPAN, estimations of potentially plant-available N based on mineralization from incubation (unpublished data).

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

Table 4. Mean Values of Total Yield, Quality Parameters, and Nitrogen Parameters of Broccoli Grown with Different Fertilizers at Two Locations in Norway (Bodø and Grimstad) in Two Consecutive Years (2009 and 2010)[a]

	N rate ($kg\ ha^{-1}$)	total yield ($Mg\ ha^{-1}$)	broccoli head wt (g)	size-discarded (% of harvested <6 cm)	harvested (% of planted)	PNC_{total} % of DM	PNC_c, eq 1[b]	PNC_c, eq 2[c]
fertilizer[d]								
NF	0	5.9 de	170 de	5.8 abc	84.8 a	2.24 c	4.55 a	3.03 a
AM	80	3.8 e	134 e	12.8 a	66.1 b	1.91 d	4.53 ab	3.05 a
AD		8.7 bc	241 bc	5.3 abc	88.3 a	2.52 bc	4.39 abcde	2.70 b
SS		7.7 cd	223 cd	2.3 bc	85.5 a	2.43 bc	4.34 de	2.61 bc
SM		7.1 cd	219 cd	5.8 abc	82.2 a	2.35 bc	4.43 abcd	2.68 ab
AM	170	2.7 e	125 e	9.9 ab	52.6 c	1.70 d	4.53 abc	3.05 a
AD		9.8 ab	292 ab	0.5 c	82.4 a	2.95 a	4.35 cde	2.62 bc
SS		9.1 bc	270 bc	3.3 bc	84.8 a	2.67 ab	4.25 ef	2.48 bc
SM		7.8 c	234 c	2.8 bc	82.0 a	2.37 bc	4.37 bcde	2.65 b
MF		12.1 a	332 a	0.4 c	88.6 a	2.91 a	4.16 f	2.32 c
year								
2009		8.6	234	6.8	88.6	2.53	4.28	2.46
2010		6.3	214	3.0	70.9	2.28	4.50	3.00
location								
Grimstad		8.7	266	2.5	83.5	2.14	4.27	2.46
Bodø		6.2	182	7.4	75.9	2.67	4.51	3.00
SEM[e]		0.33	7.96	0.789	1.63	0.0573	0.0247	0.0535
treatment		0.000	0.000	0.000	0.000	0.000	0.000	0.000
year		0.013	0.010	0.003	0.000	0.000	0.000	0.000
location		0.000	0.000	0.000	0.000	0.000	0.000	0.000
treatment × location		NS	NS	NS	NS	NS	NS	NS
treatment × year		NS	NS	NS	NS	NS	0.001	NS
year × location		NS	NS	NS	NS	0.000	0.000	0.000
treatment × year × location		NS	NS	NS	0.014	0.014	NS	NS
replication (year location)		0.012	0.015	0.001	0.009	NS	NS	NS

[a]Variables in the same column followed by similar letters are not significantly different by analysis of variance and Tukey's test ($p > 0.05$). Total yield includes broccoli of all sizes. [b]Greenwood et al., 1996.[17] [c]Greenwood et al., 1986.[16] [d]NF, no fertilizer; AM, algae meal; AD, anaerobically digested food waste; SS, shrimp shell; SM, sheep manure; MF, mineral fertilizer. [e]SEM, standard error of the mean.

(unpublished results). Organic materials and waste resources equivalent to 300 kg N ha^{-1} were homogeneously incorporated in soil (50 g of DM soil) from the field in Bodø. Soil with and without mixed-in fertilizer material was incubated (Termaks B8420S, Norway, Bergen) at 15 °C for 60 days. Soil moisture was kept at field capacity (−5 kPa) by the addition of distilled water twice a week. After 1, 10, 18, 39, and 60 days, triplicates of soil samples from each treatment were sampled and stored at −20 °C. The content of NO_3^--N and NH_4^+-N was determined by extracting 40 g of frozen sample in 200 mL of 1 M KCl prior to analysis. Fertilizer-derived inorganic N was obtained as the difference between fertilized and unfertilized soil. The fertilizer-derived N potentially available to plants was determined after an extended phase of only minor changes in measured values. The mean values measured at the last sampling were 53.9, 54.1, and 86.3% of the N, which would correspond to 300 kg ha^{-1} for SM, SS, and AD, respectively, whereas AM immobilized more N than it released (Table 3). The temperature sum at the last sampling during the incubation was 900 degree days, as compared to 823 and 697 in Bodø and 979 and 1116 in Grimstad for the growing seasons of 2009 and 1010, respectively, measured by agricultural climatic services in Norway (LMT), weather stations in Vågønes and Landvik.

Plant N Concentration. Total plant N concentration (PNC_{total}) in the above-ground part of the broccoli plant (leaf, stem, and edible part) was compared to critical plant N concentrations (PNC_c) calculated by two different equations: eq 1 specific for brassica[17] and eq 2 for arable crops in general:[16]

$$PNC_c = 5.2 - 0.178W \qquad (1)$$

where W = total DM ha^{-1} < 14.4 t ha^{-1}

$$PNC_c = 1.35 + 4.05\ e^{-0.26W} \qquad (2)$$

In these equations, W = total DM ha^{-1}.

Glucosinolate (GLS) Content. For GLS analyses, broccoli plants fertilized with SS, SM, and MF corresponding to 170 kg N ha^{-1}, and NF were chosen. The frozen powder of broccoli florets was freeze-dried (Christ Gamma 1-16, Christ, Osterode, Germany) and ground in a mortar to a fine powder before extraction. Samples for HPLC analysis were prepared according to the method of Vallejo et al.[32] and ISO 9167-1:1992,[33] with several modifications. A sample of about 200 mg of the broccoli powder was placed in a graduated 15 mL tube. The sample tubes were heated at 73 °C in water for 3 min, then 4.5 mL of preheated (73 °C) 70% methanol was added, and the samples were mixed and kept for 3 min at 73 °C. As internal standard, 100 μL of a 2.25 mM glucotropaeolin (Applichem GmbH, Darmstadt, Germany) solution was added. After 10 min at room temperature, the samples were centrifuged at 5300g for 15 min at 20 °C. The supernatant was decanted into a new tube and the pellet re-extracted with 3.0 mL of 70% methanol at room temperature and centrifuged again. The two supernatants were combined, and the extracts were stored at 4 °C until GLS desulfatation the same day. A volume of 0.5 mL of DEAE Sephadex suspension (DEAE Sephadex A-25 (GE Healthcare Biosciences AB, Uppsala, Sweden) expanded, washed twice, and suspended 1:3 (v/v) in 0.02 M sodium acetate buffer, pH 5.0) was added to a 1 mL syringe fitted with ultrafine glass wool. The column was washed with 0.5 mL of water, then 2 × 0.5 mL of sample extract was added, and the column was washed again with 2 × 0.5 mL of water. The pH was stabilized with 2 × 0.5 mL of 0.2 M sodium acetate buffer (pH 5.0) before 75 μL of purified sulfatase (25 mg mL^{-1} of *Helix pomatia* type H1, Sigma-Aldrich Co., St. Louis, MO, USA) was added. The column was kept at room temperature overnight (at least

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

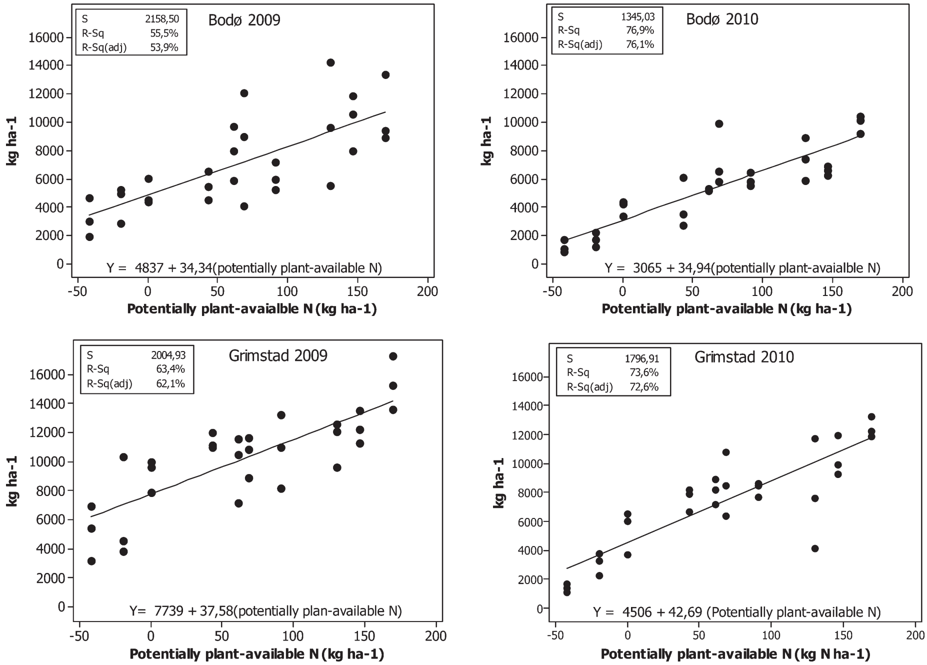

Figure 1. Broccoli yield (kg ha^{-1}) in Bodø and Grimstad in 2009 and 2010 regressed on estimated potentially plant-available N (kg ha^{-1}) for the different fertilizers. Estimates are based on mineralization data.

11 h). Desulfoglucosinolates were eluted by addition of 0.5 + 0.5 + 0.25 mL of water, and the total eluate was passed through a 0.45 μm Millex-HV PVDF filter (Merck Millipore Ltd., Cork, Ireland). HPLC analysis was carried out using an Agilent Technologies (Santa Clara, CA, USA) 1100 series system comprising a quaternary pump, an inline degasser, a thermostat-controlled (5 °C) autosampler, a column heater, and a photodiode array detector. Separation was performed on a Spherisorb ODS2 (Waters Corp., Milford, MA, USA) 5 μm 4.6 × 250 mm cartridge fitted with a Spherisorb ODS2 5 μm 4.6 × 10 mm guard column and operated at 30 °C with a flow of 1.5 mL min^{-1}, an injection volume of 30 μL, and detection at 227 nm. The mobile phases were (A) water and (B) 20% (v/v) acetonitrile, and the gradient elution program was 1% B for 1 min, linear gradient to 99% B for 20 min, 99% B for 3 min, linear gradient to 1% B for 5 min, and then 1% B for 10 min. Desulfoglucosinolates were identified by comparison of retention times and UV absorbance spectra with those of known standards and on previous mass identification by LC/Q-TOF/MS (Agilent Technologies). Concentrations were calculated from peak areas using response factors relative to glucotropaeolin (ISO 1967-1:1992) and expressed as micromoles per gram of DM.

Sensory Analysis. Prior to sensory analysis, the vacuum-packed broccoli florets were thawed at 4 °C overnight. The bags were heated with steam for 6 min at 100 °C. The assessors were served broccoli florets of 10−30 g with 2 cm of floret stem. Samples were randomized in pairs, and corresponding samples from each location were analyzed on the same day. The florets were served in preheated porcelain bowls placed on a hot plate. Within each session samples were randomized with respect to serving order. The sensory analyses were carried out during a 3-day session.

A descriptive sensory analysis was performed (ISO 6564:1985E) by a trained sensory panel of eight assessors (Nofima, Ås. Norway). Twenty-nine sensory attributes within flavor, taste, appearance, color, odor, and texture were evaluated. The sensory panel was calibrated using MF- and AM-fertilized broccoli grown in Grimstad. Appearance and color attributes were evaluated on the larger of the two florets, whereas taste, odor, flavor, and texture attributes were evaluated on an average of two florets. To assess the odor, the assessors cut the florets longitudinally. The texture was evaluated by a bite at the area between the buds and the floret stem, allowing a part of the bud and of the stem to be evaluated. The panelists recorded their results at individual speed on a 15 cm nonstructured continuous scale. The data registration system, EyeQuestion, v. 3.8.6 (Logic 8, The Netherlands) transformed the responses from 0−15 cm on the screen to numbers from 1.0 (low intensity) to 9.0 (high intensity).

Statistical Analysis. Analysis of variance (ANOVA) was performed using general linear model (GLM) in Minitab 16 (Minitab Inc., State College, PA, USA) to determine the statistical effects of design variables on the yield parameters, PNC, GLS, and sensory quality parameters. Analysis of variance was also conducted for each location and year for the different treatments. GLM analysis was performed using fertilizer treatment, location, and year as main factors, whereas interactions between main factors and replicates were nested within year and location. For the sensory analyses, the individual assessor was considered as random (main) factor, whereas the other factors were fixed. Year and session in sensory analysis were confounded. Tukey's test was used to confirm effect of individual fertilizer treatments.

Regression analysis was performed in Minitab 16 to test the relationship between estimated N from fertilizer materials potentially available to plants during the growing season and measured broccoli yield and GLS content. Pearson correlation analyses were performed to reveal possible relationships between estimated potentially plant-available N, content of total N or total S in fertilizer materials and contents of GLS and between sensory attributes and phenological expressions (yield, PNC_{total}, fresh weight, N uptake, and estimated

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

Table 5. Mean Glucosinolate Content (Micromoles per Gram DM) in Broccoli Grown at Two Locations (Bodø and Grimstad) and in Two Years (2009 and 2010) Using Fertilizers at 0 and 170 kg N ha^{-1}[a]

	N rate (kg ha^{-1})	GLS[c]	ALI	GLI	GLR	IND	4OHGLB	GLB	4MGLB	NGLB	ALI/IND	GLR/GLB	GLR/NGLB
fertilizer[b]													
NF	0	13.36 b	8.04 bc	1.06 ab	9.98 bc	5.32 c	0.16	2.11 b	0.46	2.59 b	1.77 a	3.65 a	3.81
SM	170	10.59 b	5.60 c	0.68 b	4.91 c	4.99 bc	0.15	1.90 b	0.53	2.42 ab	1.35 b	2.90 b	2.77
SS	170	23.00 a	11.41 a	1.25 a	10.16 a	11.59 a	0.16	5.09 a	0.72	5.62 a	1.08 b	2.03 b	2.42
MF	170	17.06 a	9.07 ab	1.06 ab	8.02 ab	7.99 ab	0.14	3.90 a	0.60	3.35 ab	1.28 b	2.27 b	3.03
SEM[d]		1.240	0.618	0.087	0.534	0.708	0.0232	0.284	0.048	0.413	0.0905	0.177	0.275
treatment		0.000	0.000	0.012	0.000	0.000	NS	0.000	NS	0.015	0.004	0.000	NS
year		0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.001	0.014	0.001
location		NS	0.019	0.003	0.029	NS	0.000	NS	0.001	NS	NS	NS	NS
treatment × location		NS	0.004	0.021	0.003	NS	NS	NS	NS	NS	NS	NS	NS
treatment × year		NS	NS	NS	0.110	NS	NS	NS	NS	NS	NS	NS	NS
location × year		NS	NS	NS	NS	NS	0.001	0.001	NS	NS	0.002	0.000	0.022
treatment × location × year		NS	NS	NS	NS	NS	NS	0.014	NS	NS	NS	NS	NS
replication (location year)		0.043	0.008	NS	0.006	NS	NS	NS	NS	NS	NS	NS	NS

[a]Values followed by the same letters are not significantly different ($n = 3$), Tukey's test ($P < 0.05$). [b]NF, no fertilizer; SM, sheep manure; SS, shrimp shell; MF, mineral fertilizer. [c]GLS, total glucosinolates; ALI, total aliphatic; IND, total indolic; GLI, glucoiberin; GLR, glucoraphanin; 4-OHGLB, 4-hydroxy-glucobrassicin; GLB, glucobrassicin; 4MGLB, 4-methoxyglucobrassicin; NGLB, neoglucobrassicin. [d]SEM, standard error of the mean.

potentially plant-available N). The correlation analysis was performed for results obtained both years and within each year separately.

Principal component analysis (PCA) was performed using Minitab 16 on yield and N parameters, GLS, and statistically significant sensory attributes.

■ RESULTS

Yield and Plant Nitrogen Concentrations. The yield varied in response to year, location, and fertilization (Table 4). The yield ranged from 1.2 Mg ha^{-1} (AM 170 kg N ha^{-1}, Bodø 2010) to 15.4 Mg ha^{-1} (MF 170 kg N ha^{-1}, Grimstad 2010). MF gave significantly higher yield than all other fertilizer treatments except for AD supplied at the rate of 170 kg N ha^{-1}. AM produced yields that were significantly lower compared to the other fertilizer materials at both N rates and were at similar levels as for NF. There were no significant differences in yield between AD, SS, and SM at a fertilizer rate of 80 kg N ha^{-1}, but at 170 kg N ha^{-1} AD gave higher yield than SM. Differences were visible as distinct differences in plant size, leaf area, and plant height. In Grimstad in 2009, symptoms of N deficiency were observed as broccoli heads tended to be yellowish or violet and poorly developed with high compactness and only single buds reaching maturity. These quality disorders were registered by the sensory panel as degree of uniformity in bud size and color.

The mean PNC_{total} over year and location ranged from 1.7 to 3.0% (Table 4). Significantly higher PNC_{total} was observed in broccoli fertilized with AD and MF and significantly lower PNC_{total} for broccoli fertilized with AM. PNC_c ranged from 4.2 to 4.6% when calculated by eq 1 and from 2.3 to 3.1% when calculated by eq 2. The PNC_c calculated by eq 1 was considerably higher than all PNC_{total}.

The PNC_{total} was higher than PNC_c calculated by eq 2 in 3 of the 10 fertilizer treatments, and these were AD and SS at s rate of 170 kg ha^{-1} and MF.

Total yield was linearly correlated with estimated amount of inorganic N potentially available from the fertilizer materials during the growing season (Figure 1).

Glucosinolates. The total GLS content was significantly higher for broccoli fertilized with SS and MF (23.0 and 17.1 μmol g^{-1} DM, respectively) (Table 5). These fertilizer materials provide an estimated plant-available N during the growing season corresponding to 92 and 170 kg N ha^{-1} and a high S content of 83 and 81 kg S ha^{-1} for SS and MF, respectively. In contrast, total GLS content in broccoli after SM and NF treatment was significantly lower (11.6 and 13.4 μmol g^{-1} DM, respectively) (Table 5), even though SM corresponds to a plant-available N content of 92 kg ha^{-1} and an S content of 23 kg ha^{-1}. Aliphatic GLS represented 48.3% (SM) to 59.7% (NF) of total GLS content, whereas the indolic GLS represented 39.6% (NF) to 50.4% (SS). Both total aliphatic and total indolic GLS contents were significantly higher in broccoli fertilized with SS compared to SM and NF. Neither total N nor estimated potentially plant-available N derived from fertilizer materials during the growing season correlated with total GLS, total aliphatic, or total indolic GLS content. However, when each year was analyzed separately, correlations between total N or estimated potentially plant-available N and total indolic GLS were found in 2009 (correlation coefficients of 0.504 and 0.451, respectively; $p < 0.05$). Correlations were found between S content in added fertilizer materials and total GLS, total aliphatic GLS, and total indolic GLS (correlation coefficients of 0.463, 0.362, and 0.495, respectively; $p < 0.05$). Total GLS content was 84.1% higher in 2010 than in 2009. Glucoraphanin was the main aliphatic GLS and constituted on average 88.3% of total aliphatic GLS. Glucoraphanin level was significantly lower for SM compared to SS and MF and correlated with S content and N:S ratio in fertilizer (0.389 and −0.320, respectively; $p < 0.05$). Among the individual indolic GLS, differences between fertilizer treatments were observed for glucobrassicin and neoglucobrassicin, which were the main indolic GLSs (on average 43.8 and 46.8%, respectively, of total indolic GLS content). Glucobrassicin was significantly higher for SS and MF and correlated with total amount of N, estimated potentially plant-available N from fertilizer materials, and S content (correlation coefficients of 0.378, 0.372, and 0.659, respectively; $p < 0.05$). A significantly higher level of neoglucobrassicin was found for SS when compared to NF, and neoglucobrassicin content correlated with S content (correla-

DOI: 10.1021/acs.jafc.5b04631
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Table 6. Numeric Assessment (from 1 to 9) of Selected Sensory Attributes of Broccoli Grown with Different Fertilizers in Two Years (2009 and 2010) at Two Locations in Norway (Bodø and Grimstad)[a]

	N rate (kg ha^{-1})	uniform bud size	whiteness	violet color	firmness	crispness	juiciness	astringency	fibrousness	sour odor	bitter odor	sulfur odor	sour taste	salty taste	sulfur taste	water taste	after taste
fertilizer[b]																	
NF	0	6.37 ab	3.41 a	1.15 ab	3.61 ab	3.58 cd	5.21 ab	2.00 cd	2.35 c	3.58 a	3.92 ab	3.46 b	3.63 a	1.46 bc	3.64 ab	1.97 ab	4.59 cd
AM	80	5.80 b	3.21 abcd	1.15 ab	3.35 b	4.08 abcd	5.18 ab	2.59 ab	2.8 abc	2.98 bc	3.36 cde	3.74 ab	2.90 b	1.72 ab	3.83 ab	2.32 a	5.21 ab
AD		6.02 ab	2.88 d	1.30 a	3.30 b	4.07 abc	5.07 ab	2.63 a	2.90 abc	2.78 c	3.46 bcde	3.77 ab	2.81 b	1.66 abc	3.89 a	2.25 ab	5.20 ab
SS		6.09 ab	2.94 bcd	1.30 ab	3.69 ab	4.45 a	5.42 a	2.43 abc	2.92 ab	3.26 abc	3.40 be	3.63 ab	3.31 ab	1.69 ab	3.43 b	1.88 ab	5.03 abc
SM		6.04 ab	3.26 abcd	1.32 a	3.53 b	4.01 abcd	5.08 ab	2.64 a	3.00 a	2.91 bc	3.45 bcde	3.91 a	2.91 b	1.71 a	3.88 ab	2.13 ab	5.27 a
AM	170	5.90 ab	3.52 a	1.16 ab	3.51 ab	3.35 de	4.97 ab	2.05 bcd	2.56 abc	3.25 abc	3.93 abcde	3.74 b	3.31 ab	1.50 abc	3.89 ab	2.20 ab	4.61 bcd
AD		6.28 ab	3.35 abc	1.14 ab	3.41 b	3.32 e	5.09 ab	2.01 cd	2.39 bc	3.29 abc	3.89 abcde	3.60 ab	3.33 ab	1.43 c	3.77 ab	2.30 a	4.60 cd
SS		6.49 a	3.36 ab	1.16 ab	3.99 a	3.99 abcd	5.26 ab	1.90 d	2.77 abc	3.62 a	3.83 abcde	3.44 b	3.66	1.48 abc	3.54 ab	1.76 b	4.53 d
SM		6.49 a	3.56 a	1.11 b	3.69 ab	3.62 bcde	4.96 b	2.02 cd	2.69 abc	3.39 ab	3.99 a	3.61 ab	3.35 ab	1.47 abc	3.78 ab	2.06 ab	4.65 cd
MF		6.05 ab	2.93 cd	1.17 ab	3.36 b	4.16 ab	5.29 ab	2.41 abc	2.68 abc	3.09 abc	3.41 e	3.71 ab	3.15 ab	1.70 ab	3.67 ab	2.11 ab	5.15 ab
SEM[c]		0.0471	0.0325	0.0142	0.0332	0.0434	0.0318	0.0346	0.0395	0.0410	0.0387	0.0326	0.0417	0.0180	0.0326	0.0372	0.0359
treatment		0.006	0.000	0.000	0.000	0.000	0.036	0.000	0.002	0.000	0.000	0.038	0.000	0.000	0.023	0.007	0.000
year/session		0.000	0.000	0.000	0.000	0.000	0.001	0.000	NS	NS	0.000	0.000	0.022	0.001	0.000	0.000	0.000
location		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.023	NS	NS	0.047	NS	NS
panelist		0.035	NS	0.001	NS	0.029	0.039	0.000	0.000	0.000	NS	0.054	0.045	NS	0.008	0.000	NS
treatment × year		0.051	0.006	0.000	NS	0.000	NS	NS	NS	NS	0.001	NS	NS	NS	NS	NS	NS
treatment × location		NS	NS	0.000	0.010	0.017	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
year × location		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
replication (year location)		NS	NS	0.003	0.000	0.011	NS	NS	0.000	0.005	NS	NS	NS	NS	NS	NS	NS

[a]Variables in the same column followed by similar letters are not significantly different by analysis of variance and Tukey test ($p > 0.05$). [b]NF, no fertilizer; AM, algae meal; AD, anaerobically digested food waste; SS, shrimp shell; SM, sheep manure; MF, mineral fertilizer. [c]SEM, standard error of the mean.

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

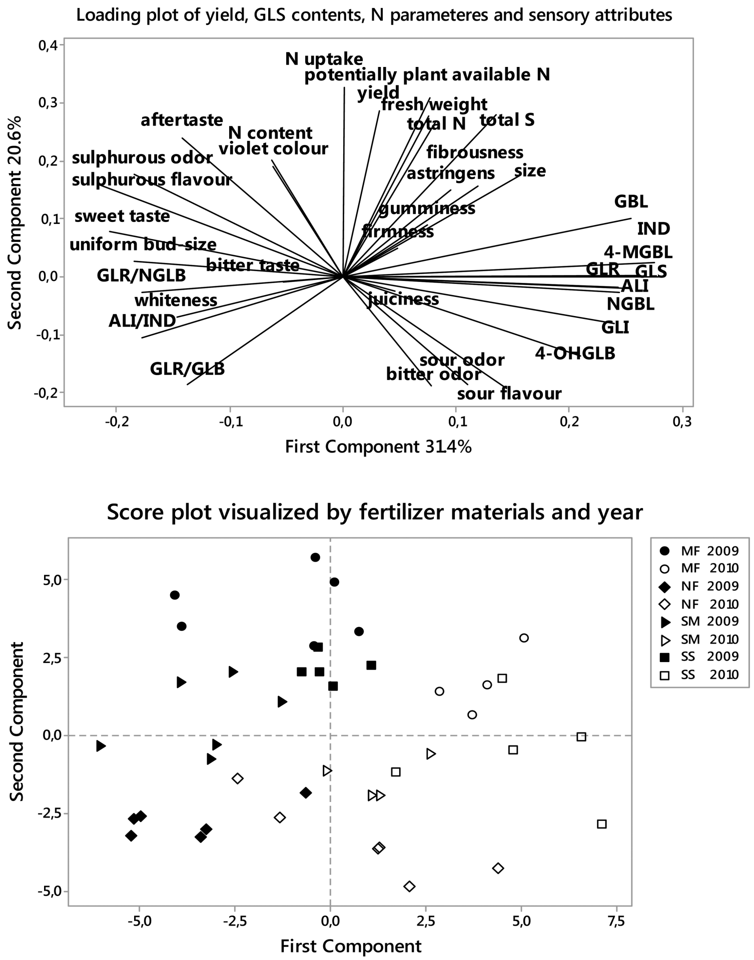

Figure 2. Loading plot and score plots from PCA of broccoli grown with different fertilizer materials at a southern (Grimstad) and a northern location (Bodø) for two years (2009 and 2010). The first two principal components explain 52.0% of the variation in GLS content, N and phenological parameters, and sensory attributes. Fertilizer ID abbreviations: NF, no fertilizer; MF, mineral fertilizer; SM, sheep manure; SS, shrimp shell; GLS, total glucosinolates; ALI, total aliphatic; IND, total indolic; GLI, glucoiberin; GLR, glucoraphanin; 4-OHGLB, 4-hydroxy-lucobrassicin; GLB, glucobrassicin; 4MGLB, 4-methoxyglucobrassicin; NGLB, neoglucobrassicin.

tion coefficient of 0.365; $p < 0.05$) and correlated with N content or estimated potentially plant-available N in year 2009 (correlation coefficients of 0.483 and 0.436, respectively; $p < 0.05$). Aliphatic GLS level is significantly higher in Grimstad than in Bodø.

The ratio between aliphatic and indolic GLS and the ratio between glucoraphanin and glucobrassicin varied with fertilizer treatment and year (Table 5) and were correlated with estimated potentially plant-available N (correlation coefficients of −0.338 and −0.468, respectively; $p < 0.05$), total amount of N (correlation coefficients of −0.417 and −0.500, respectively; $p < 0.05$), and total S content (correlation coefficients of 0.396 and 0.554, respectively; $p < 0.05$) in added fertilizer materials. The ratio between glucoraphanin and neoglucobrassicin was not influenced by fertilizer treatment, but was influenced by year.

Sensory Quality. Significant effects of fertilizer materials were observed for 16 of 29 sensory attributes evaluated (Table 6); however, there were no obvious trends in how sensory attributes were influenced. The differences in sensory score for the individual attributes were from 2.2 to 12.2%. In general, sensory attributes were not influenced by location. However, higher levels of sulfur odor and taste were found in Bodø, and higher levels of green odor and taste were found in Grimstad (data not shown).

Sensory attributes were correlated with neither estimated potentially plant-available N nor other phenological expressions

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

such as yield, PNC_{total}, fresh weight, or N uptake (data not shown).

Principal Component Analysis. The PCA of yield, sensory attributes, contents of GLSs, and N parameters for fertilization material, location, and year shows that 52.0% of the variation could be explained by principal components one and two (Figure 2). In the score plot visualized by fertilizer materials and year, the strongest factor for variable grouping seems to be year. For yield, GLS, and N parameters, the year factor is mainly explained by the climate effect. However, for sensory attributes, the climate effect is confounded by possible differences between sensory sessions performed for different years. The score plots show a tendency to grouping by year in two groups. The 2010 samples were located in the right part of the score plot and characterized with high content of GLSs, bitter odor, sour flavor, and sour odor. The 2009 samples were located in the left part of score plot and mainly associated with high tendency to uniform bud size, high N content, high score for aftertaste, salty taste, violet color, sulfur flavor and sulfur odor, water flavor, and whiteness. A score plot for fertilizer materials shows grouping tendency; however, there was overlap between source. MF and NF samples were clearly separated in the upper and lower parts of the score plot, respectively, with the other fertilizer materials in an intermediate position. MF was associated with high yield, N content, size, fresh weight, and GLS content and high scores for salty taste, aftertaste, violet color, crispness, firmness, and sulfur odor. NF samples were associated with sour odor, sour flavor, bitter odor, and whiteness as well as high glucoraphanin/glucobrassicin ratio and aliphatic/indolic GLS ratio. Furthermore, broccoli fertilized with SM was associated with high score for uniform bud size and whiteness. Broccoli fertilized with SS was associated with the same sensory attributes as MF, but had a stronger association with the different GLS.

■ DISCUSSION

Yield and Plant N Concentration. The linear correlation between broccoli yield and estimated potentially plant-available N during the growing season, with no diminishing return, suggests that the optimum N supply was not reached at a rate of 170 kg N ha^{-1}. This is supported by the PNC_{total} being below PNC_c for brassicas (eq 1), indicating that the N availability was suboptimal even for the fertilizer material with the highest N-supplying potential. However, calculating PNC_c by eq 2 for arable crops indicates that broccoli fertilized with SS and AD at 170 kg N ha^{-1} and MF reached the optimum, as PNC_{total} values were below PNC_c. The model defining PNC_c for brassica (eq 1) has previously been found to overestimate the content of N, whereas PNC_c estimated by eq 2 for arable crops fits experimental data better or even underestimates.[17,34] The N fertilizer rate at 170 kg N ha^{-1} is the upper limit for average N supply rate on arable land in organic farming in Norway. This rate is, however, below the recommended N fertilizer rate for conventional broccoli production in Norway, which is 200−250 kg N ha^{-1}, assuming an average marketable yield of 8−10 Mg ha^{-1}.[35] Considering the N mineralization from soils and the organic fertilizers' N, the yields in the present study are as expected. This result is in agreement with previous studies showing that N is a growth-limiting nutrient in broccoli production.[36−38]

The similarity of recorded yield and PNC_{total} values obtained for broccoli fertilized with SS and AD at the high N rate and those obtained with MF (Table 4) suggests that these fertilizers, when supplied according to the Norwegian regulation for organic agriculture,[39] may offer an adequate amount and timing of supply of N to meet the demand of broccoli. In contrast, N fertilization with SM and AM was clearly insufficient, which can be explained by different biochemical compositions, notably resulting in higher C:N ratios and, consequently, lower net N mineralization potential (Table 3). In AD, 70% of the N was inorganic and thus potentially plant-available at application time (data not shown). During incubation in soil at 15 °C for 60 days, another 15% of the N was mineralized. On the other hand, for AM there was no net N immobilization during the incubation, which explains the negative fertilizer effect in the present study. This is consistent with the observed linear relationships between potentially plant-available N and yield.

Significant differences found for year and location may be due to climatic conditions. In Bodø, it is likely that the differences in yield between years was influenced by a 1.8 °C lower average temperature and a substantially lower number of sunshine hours in 2010 than in 2009, which may affect N mineralization in soil as well as broccoli plant growth and development.[37,40,41] In addition, above normal precipitation in 2010, especially around transplanting and during the first weeks of plant development, may have resulted in NO_3^- leaching, and consequently, contributed to the lower N uptake in 2010. In Grimstad, temperature or sunshine hours cannot explain the difference between years, but precipitation may explain the different broccoli size and color.

Glucosinolates. The content of GLS was influenced by type of fertilization. The availability of N and S and the N:S ratio has previously been shown to influence the content of GLS.[18,24,25,42] In the present study neither total N supply, estimated as potentially plant-available N, nor N:S ratio correlated with total GLS content; however, there was a positive correlation between total GLS content in broccoli and S content in fertilizer materials. The high total GLS level in broccoli fertilized with SS and MF, which had the highest S content among the fertilizer materials, and the low level of total GLS in broccoli fertilized with SM with low S content indicate that S supply might be more important for the total GLS content than N supply and N:S ratio at the current fertilizer rates. This is in accordance with previous studies in which increasing S supply results in higher total GLS content.[43−45] Li et al.[43] found that increasing N fertilization at high S fertilizer rate did not affect the total GLS content, and Vallejo et al.[32] found no differences in total GLS content in broccoli fertilized with increasing N supply (15−150 kg N ha^{-1}). However, the high content of the indolic GLS glucobrassicin in broccoli fertilized with SS and MF compared with SM and NF might be explained by N levels during the growing period as there were correlations between the content of glucobrassicin and both the estimated plant-available N and total N added. These results are in agreement with results obtained for vegetable turnip rape (*Brassica rapa* L.), for which the GLS content increased with increasing N regardless of S supply.[24] The higher aliphatic:indolic ratio in broccoli receiving NF is in accordance with previous results, where an increase in indolic GLS and a decrease in aliphatic GLS with increasing N supply have been found.[25,46,47] Consequently, the higher content of total GLS content in broccoli fertilized with SS and MF cannot, solely, be explained by variation in the nutritional status for N, but must also be seen in relation to S status and the ratio between N and S.

DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

The high content of GLS in broccoli fertilized with SS might also be due to the content of chitin in shrimp shells. Chitin in SS is the same as chitin found in insect herbivores and may in plants induce stress responses that can influence biosynthesis of GLS, which are phytochemicals important in plant defense.[26]

The higher aliphatic GLS level in Grimstad compared to Bodø is in accordance with the results of Steindal et al.,[48] who found highest aliphatic GLS level in broccoli grown at high temperature in combination with 12 h of daylight.

Sensory Attributes. The present study showed only minor effects of fertilizer material and N rate on sensory attributes of broccoli. Some of the differences in sensory attributes may be explained as indirect effects of the applied fertilizers on plant development stage, which have been found to influence sensory attributes,[49] rather than direct effects of fertilizer on the sensory properties per se. In this study, many broccoli plants fertilized with AM never reached maturity, and the plants appeared very small with a high degree of gumminess even at a premature stage. Broccoli fertilized with easily available N matured more evenly, which is in agreement with known effects of N availability on growth and development stage.[37,40,41] Differences in sensory attributes of vegetables grown organically and conventionally show inconsistent results.[27,28]

The overall PCA plot showed that year was the most important factor explaining the variation in the samples.

In conclusion, broccoli yield and contents of N and GLS were significantly influenced by type of fertilizer source. Yield increased linearly with estimated potentially plant-available N during the growing season, which resulted in the following yield order: MF > AD > SS > SM > NF > AM. No such linear relationship was found for the GLS content. However, application of SS and MF gave higher contents of some GLS than fertilization with SM and NF. Sensory attributes were more influenced by sensory session (year) than by fertilizer material and location. This study showed that in terms of broccoli crop development and yield, further research on the use of organic and waste-derived fertilizers should focus on the determination and prediction of fertilizer-derived plant-available N. When it comes to effects on GLS content, the results suggest a response to the N and S status in fertilizer materials, but more work needs to be done to determine the causes of the measured effects of certain fertilizers. Relatively little is known about the effects of climate and other site-specific factors on GLS concentration, which makes it a substantial challenge experimentally to separate fertilizer-specific causal factors from those varying more erratically such as temperature and precipitation.

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Funding

We are grateful to the Research Council of Norway, the counties of Nordland and Troms, and the Norwegian Institute for Agricultural and Environmental Research for financial support of this work.

Notes

The authors declare no competing financial interest.

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DOI: 10.1021/acs.jafc.5b04631
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DOI: 10.1021/acs.jafc.5b04631
J. Agric. Food Chem. 2015, 63, 10757−10767

Paper II

Øvsthus I, Seljåsen R, Stockdale E, Uhlig C, Torp T, Breland TA, 2017. Yield, nitrogen recovery efficiency and quality of vegetables grown with organic waste-derived fertilisers. Nutrient Cycling in Agroecosystems 109(3):233–248

Nutr Cycl Agroecosyst (2017) 109:233–248
DOI 10.1007/s10705-017-9881-7

ORIGINAL ARTICLE

Yield, nitrogen recovery efficiency and quality of vegetables grown with organic waste-derived fertilisers

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Received: 1 February 2017 / Accepted: 15 September 2017 / Published online: 30 September 2017

Abstract More sustainable production of high-quality, nutritious food is of worldwide interest. Increasing nutrient recycling into food systems is a step in this direction. The objective of the present study was to determine nitrogen (N) fertiliser effects of four waste-derived and organic materials in a cropping sequence of broccoli, potato and lettuce grown at two latitudes (58° and 67°N) in Norway during 3 years. Effects of anaerobically digested food waste (AD), shrimp shell (SS), algae meal (AM) and sheep manure (SM) at different N application rates (80 and 170 kg N ha^{-1} for broccoli, and 80 and 60 kg N ha^{-1} for potato and lettuce, respectively) and residual effects were tested on crop yield, N uptake, N recovery efficiency (NRE), N balance, N content in produce, mineral N in soil, product quality parameters and content of nitrate in lettuce. Mineral fertiliser (MF) served as control. Effects on yield, N uptake, NRE, N balance and product quality parameters could to a great extent be explained by estimated potentially plant-available N, which ranked in the order of AD > SS > SM > AM. Results for crops fertilised with AD and SS were not significantly different from MF at the same N application rate, while AM, in agreement with its negative effect on N mineralisation, gave negative or near-neutral effects compared to the control. No residual effect was detected after the year of application. The results showed that knowledge about N dynamics of relevant organic waste-derived fertilisers is necessary to decide on the timing and rate of application.

Keywords Organic fertiliser · Broccoli · Potato · Lettuce · Nitrogen use efficiency · Vegetable quality

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Introduction

In agriculture and horticulture, a major aim is cost-efficient production of sufficient high-quality, nutritious food without health hazards and contaminants and with minimum detrimental impact on the environment. In organic production systems, this is pursued through the design and management of locally adapted agroecosystems in accordance with ecological principles (IFOAM 2014). The cycling and supply of nutrients to support crop growth is essential and often a main focus of farm management practice (Gliessman 2007); the organic farming standards require that operators "shall return nutrients, organic matter and other resources removed from the soil through harvesting by the recycling, regeneration and addition of organic materials and nutrients" (IFOAM 2014). These approaches are also among the solutions suggested to mitigate potassium deficiency in some soils and agricultural systems (Öborn et al. 2005) and to meet the global challenge of increasing phosphorus demand and decreasing rock phosphate availability within a few decades (Cordell et al. 2009). Currently, however, nitrogen (N) is most often the growth-limiting nutrient (Mosier et al. 2004; Zebarth et al. 1995), particularly in organically grown cash crops (Berry et al. 2002). In such systems, which are often on stockless farms, the limitation is partly due to scarcity of traditional resources, such as animal manure, and costs related to setting aside field area for green manure production in combination with too short growing season for both cash crop and manuring crops. Poor N use efficiency (NUE) due to microbial immobilisation and humification and to poor synchrony of fertiliser N mineralisation and nutrient uptake of the crop, can lead to reduced crop yield and also result in N loss to the environment by gas emission or leaching (Huggins and Pan 2003). The applied N taken up by the produce is commonly expressed as N recovery efficiency (NRE, Cassman et al. 2002; Crasswell and Godwin 1984; Fixen 2005; Mosier et al. 2004; Raun and Johnson 1999). As NUE tends to be high when N input rate is low, an important objective is to improve the NUE without reducing the productivity and quality of the produce (Roberts 2008). Additionally, if mineralisation occurs too late in the growing period, undesirably high concentrations of nitrate (NO_3^-) in leafy vegetables may occur. Overall N scarcity and poor synchrony are likely to occur when growing vegetables, e.g., *Brassica* spp., that have high N demands (Nkoa et al. 2003), especially within the arctic circle, where the growing season is short and N mineralisation from soil organic matter may be severely limited by low soil temperatures. This definitely represents a bottleneck to obtaining acceptable yields of sufficient quality (Machado et al. 2010).

Consequently, to increase the current production of organic crops and to meet the anticipated challenges of global food production in a sustainable and economic way, there is a need to investigate the fertiliser value of potential organic nutrient resources. Ideally, local resources should be used, considering the environmental costs of transportation. In Norway, there are from agriculture, aquaculture and household organic wastes or by-products that are relevant as fertilisers. The organic food waste sorted out from household wastes amounted to 180,000 Mg in 2015 (personal communication, Statistics Norway's Information Centre, Oslo, Norway). This material can potentially be utilised as fertiliser either from compost or from by-product of biogas production (RVF-Utveckling Utveckling 2005). From fish industry, registered amounts of organic waste in 2012 was 816,500 Mg, including wastes from cod and herring offshore fishing, fish farming, shrimp and crab industry (RUBIN 2012). According to RUBIN (2012), 77% of by-products from fish industry are being utilised. Waste from shrimp industry amounts to 4500 Mg, which gives a utilisation rate of 50%. As the aquaculture industry currently is growing, the potential amount of organic waste from fish is increasing. In addition to the given numbers, there are large unrecorded amounts of nutrients flowing as feed waste and excrements into the areas surrounding aquaculture cages. Seaweeds are relevant for capturing nutrients in fish farms (bioremediation and integrated multi-trophic aquaculture, Reid et al. 2013). Seaweeds can be harvested and utilised for feed, bioethanol fermentation and for energy production by biogas digestion (Roesijadi et al. 2010). Residues from biogas production, as well as the seaweeds itself, can be utilised for agricultural purposes as fertiliser or soil conditioner. To utilise such materials in agriculture, knowledge is needed to design sustainable, integrated bioenergy and nutrient recycling systems (Barrington et al. 2009).

The aim of the present study was to determine the fertiliser value of four locally-sourced organic materials in a cropping sequence of broccoli, potato and lettuce. The fertiliser materials tested were solid sheep manure (SM) from a local farmer, extruded shrimp shell (SS), anaerobically digested food waste from biogas production (AD), and a commercially available algae meal product (AM) originating from *Ascophyllum nodosum*. The effects on crop yield, N uptake, NRE of applied N, N balance and selected crop quality parameters were determined. Relationships between estimated potentially plant-available N and, respectively, yield, N uptake, N content in produce, NRE and selected quality parameters were investigated. Control plots of none fertiliser (NF) and mineral fertiliser (MF) were included.

Materials and methods

Site description, soil properties and weather data

The experimental fields were located at the Norwegian Institute of Bioeconomy Research, Division Bodø (Northern Norway, 67°28′N, 14°45′E) and Division Landvik, Grimstad (Southern Norway, 58°34′N, 8°52′E) during the growing seasons of 2008, 2009 and 2010. Detailed information about soil properties, cropping history and tillage prior to the experiment, and meteorological data are described by Øvsthus et al. (2015). In brief, the field in Bodø was a sandy orthic humo-ferric podzol (Haraldsen 1989), while the field in Grimstad was a gleyed sombric brunisol (Hole and Solbakken 1986) with a southwest-facing slope of 2–4% and 2–6%, respectively. Details about nutritional status of soil are summarised in Table 1. Prior to cropping experiment, the fields were, respectively, managed as organic cattle pasture and organic grass seed ley. From June to September in 2009 in Bodø and Grimstad, respectively, average temperature was 12.2 and 15.2 °C, sum rainfall 482 and 474 mm, and sum sunshine hours 762 and 894 h. The corresponding figures in 2010 were 11.0 and 15.0 °C, 299 and 351 mm, and 634 and 909 h, respectively.

Design and management of the field experiments

A factorial field experiment with fertiliser materials (AD, SS, SM, AM, MF and NF), nitrogen (N) application rates, and additive fertiliser and crop rotation effects as independent variables, was established in an experiment with a crop rotation of broccoli (first-year crop), potato (second-year crop) and lettuce (third-year crop), as presented in Table 2. Details about nutritional status of fertiliser materials are presented by Øvsthus et al. (2015) and are summarised in Table 3. Each of three blocks was split in three large plots (30 m × 5.6 m and 30 m × 6.4 m in Bodø and Grimstad, respectively), of which one each year served as the starting point of the crop sequence; i.e., broccoli was present on one of the three large plots in each of the three years, potato in two and lettuce in one year. The three large plots were divided into ten sub-plots (6 × 2.8 m and 6 × 3.2 m in Bodø and Grimstad, respectively) for the combinations of fertiliser type, rate and residual effect. The treatments on sub-plots were randomised within each block.

Fertiliser materials were broadcast by hand. Incorporation of fertiliser materials on broccoli plots were done as described by Øvsthus et al. (2015). In 2009, all organic fertiliser was incorporated before planting broccoli and potato. For MF, 50 and 75% of the total amount was supplied prior to planting, and the remaining 50 and 25% was supplied twice and once during the growing season of broccoli and potato, respectively. In 2010, all fertilisers were applied split in the same way as MF, except AM, all of which was incorporated before planting. On broccoli plots, the second and third application took place 3 and 5 weeks after planting. On potato plots, the second fertiliser application took place when the haulm reached 0.1 m height. On lettuce plots, all fertilisers were applied before planting. For all crops, fertiliser applied before planting was worked into the soil by rotary harrowing. Fertilisers top-dressed during the growing season were not incorporated. In dry periods, a rotary broadcaster was used for irrigation.

Table 1 Chemical properties and texture of the upper 0.3 m soil layer of the experimental fields in Bodø and Grimstad (samples taken in spring 2008)

Location	Chemical properties						Texture		
	pH*	TC** ($g\ kg^{-1}$)	TN*** ($g\ kg^{-1}$)	NO_3^--N ($mg\ kg^{-1}$)	NH_4^+-N ($mg\ kg^{-1}$)	TP**** ($mg\ kg^{-1}$)	Sand	Silt	Clay
Bodø	6.1	21	1.7	7.0	3.9	840	91	7	2
Grimstad	5.9	30	1.6	11.1	1.2	790	87	10	3

* pH in water

** TC = total carbon

*** TN = total nitrogen

**** TP = total phosphorus

Table 2 Cropping system, type of fertiliser and application amounts (kg N ha^{-1}) for the ten different treatment combinations in field trials

Treatment combination codes	Fertiliser codes	1st year crop: broccoli (N, kg ha^{-1})	2nd year crop: potato (N, kg ha^{-1})	3rd year crop: lettuce (N, kg ha^{-1})
AD1	AD	80	80	0
AD2	AD	170	0	60
SS1	SS	80	80	0
SS2	SS	170	0	60
SM1	SM	80	80	0
SM2	SM	170	0	60
AM1	AM	80	80	0
AM2	AM	170	0	60
MF	MF	170	80	60
NF	NF	0	0	0

Abbreviation used for fertiliser codes are *AD* anaerobically digested food waste, *SS* extruded shrimp shell, *SM* sheep manure, *AM* algae meal, *NF* no fertiliser applied, *MF* mineral fertiliser

The production of the seedlings of broccoli *(Brassica oleracea* L. var. *italica* **cv**. Marathon*)* are described by Øvsthus et al. (2015). Seedlings of lettuce (*Lactuce sativa L. cultivar 'Ametist'* and *Lactuce sativa* L. *cultivar 'Argentinas'*) were produced by the same method as seedlings of broccoli by using organic peat-based compost, organic chicken manure and plugtrays. The mother tubers of potato (*Solanum tuberosum* L. *cv. 'Troll'*) were chitted at 15 °C for 6 weeks before planting. Broccoli and potato were planted with 18 plants in each row and 4 rows on each sub-plot. The planting distance was 0.33 m, the row space was 0.7 m, and the tramline spacing was 0.7 and 0.8 m in Bodø and Grimstad, respectively. The lettuce cultivars 'Ametyst' and 'Argentinas' were planted on biodegradable film (Orlemans plastic B. V., Genderen, The Netherlands) in beds of four and five rows in Grimstad and Bodø, respectively. Each lettuce plot consisted of two beds, and in total there were eight and ten rows per plot in Grimstad and Bodø, respectively. The plant distances within rows were 0.4 m, giving in total 120 lettuce plants on each plot in Grimstad and 150 in Bodø. 'Ametyst' and 'Argentinas' were planted in every other row. Two different cultivars were chosen due to expectations of possible unequal development conditions in different climates. In Grimstad 'Argentinas' reached maturity first and was selected as the earliest variety at this location. In Bodø 'Argentinas' grew more slowly and was outperformed by 'Ametyst', which was selected as the best variety for this location. The results presented are for the cultivar first reaching maturity on each location.

In the first year of the field experiment, broccoli was planted on biodegradable film based on corn starch (BioAgri, BioBag Norge AS, Askim, Norway) with the aim to reduce leaching and prevent weed growth. Due to problems with dissolution and mineralisation of fertilisers in the upper soil layers close to the biofilm, this practice was abandoned in the following years. Moreover, the results for broccoli in 2008 were considered atypical as compared to those in 2009 and 2010. Therefore, results obtained in 2008 were not included in the average values presented.

Monitoring sampling and analysis

To avoid edge effect, the first plant in each row was not sampled, and soil was sampled at a distance larger than 0.33 m from the plot boundary. Soil samples were collected from two soil depths (0–0.3 and 0.3 –0.6 m). In the spring prior to producing broccoli the first year, the average soil mineral N content in Bodø and Grimstad, respectively, was 22.8 and 20.1 kg N ha^{-1} in the 0–0.3 m soil layer and 8.5 and 6.1 kg N ha^{-1} in the 0.3–0.6 m layer. Further sampling was done in spring, between tillage and planting, and once after harvest. On each sub-plot, 6–10 soil cores were randomly collected, mixed by hand, and a composite sample from each depth and each sub-plot was stored at

Table 3 Chemical and physical properties of anaerobically digested food waste (AD), extruded shrimp shell (SS), sheep manure (SM) and algae meal (AM)

Fertiliser codes	Chemical properties											Physical properties
	pH*	DM %	TOC ($g\ kg^{-1}$ DM)	TKN ($g\ kg^{-1}$ DM)	NH_4^+–N ($g\ kg^{-1}$ DM)	NO_3^-–N ($g\ kg^{-1}$ DM)	C:N ratio	PPAN (%)**	P ($g\ kg^{-1}$ DM)	K ($g\ kg^{-1}$ DM)	S ($g\ kg^{-1}$ DM)	
AD	8.6	1.3	307	254	153	0	1.2	86.3	18	106	8	Liquid part
SS	9.2	90.2	301	72	0	0	4.2	54.1	27	1	4	Dried and pelleted
SM	8.8	19.4	396	37	13	0	17.4	53.9	9	22	5	Solid part, containing traces of straw
AM	6.0	89.1	406	11	0	0	36.9	−24.5	1	16	26	Dried and crushed seaweed, mainly *Ascophyllum nodolus*

* pH in water

** *PPAN* Potentially plant-available N during the growing season as estimated by Øvsthus et al. (2015) from results obtained by Øvsthus et al. (manuscript in preparation) during incubation of the fertilisers in soil at controlled temperature and moisture

– 18 °C until analysis of inorganic N. NH_4^+ and NO_3^- were determined at Norwegian Institute of Bioeconomy Research (NIBIO, location Apelsvoll, Kapp, Norway) by extraction of 40 g soil in 200 ml 1 M KCl and analysis by a Flow Injection Analyser (FIAstar 5000, Foss Analytical AB, Sweden).

For broccoli, harvesting criteria and determination of yield, quality and N content are described by Øvsthus et al. (2015).

For potato, height of the haulm was monitored in the beginning of September. Potato haulm and tuber of ten plants on each sub-plot were harvested separately in the end of September and used for analyses. The remaining sub-plots were harvested for determination of total yield. Haulm and tubers were weighed, and tubers were counted and their size recorded before they were milled in a meat grinder and dried at 60 °C for determination of dry weight (DW) and Kjeldahl N, as described for broccoli by Øvsthus et al. (2015). Reduced quality (green tuber, hollow heart and crack growth) and percentage tubers smaller than first-class size (< 40 mm) were recorded.

For lettuce, a random selection of 20–30 heads from each sub-plot were harvested when 80% of the plants had reached maturity stage, resulting in three different harvest dates depending on fertiliser treatment. Average weight per lettuce head was determined and the results computed as total yield per hectare without consideration of the number of lettuce plants that died or did not reach maturity, and that some treatments resulted in bigger heads than what is usually considered as harvesting stage. For determination of DW and Kjeldahl-N, 6–10 randomly chosen plants from each sub-plot were homogeneously milled and mixed in a meat grinder, samples of about 20 g were frozen at −18 °C and a sub-sample of about 500 g was dried at 60 °C and weighed. NO_3^- was determined by extraction of 20 g frozen sample in 100 ml boiling water, and analysis by spectrophotometry using a FIAstar 5000 Analyzer (Foss Analytical AB, Sweden). Quality parameters and size class were recorded according to NS 2830.

Apparent N recovery efficiency and N balance

Apparent nitrogen recovery efficiency (NRE) of the fertilisers was calculated as given by Crasswell and Godwin (1984).

$$\text{NRE} = (\text{U}-\text{U}_0)/\text{N}_\text{A} \quad (1)$$

where U and U_0 are uptake of N (kg ha^{-1}) in aboveground plant biomass (including content of N in potato tubers) with and without fertiliser, respectively, and N_A is the amount of N applied (kg ha^{-1}). N balance (NB) is the difference between accumulated input and output after 1–3 years, respectively.

$$\text{NB} = \text{N}_\text{A} - \text{N}_\text{Y} \quad (2)$$

where N_Y is the amount of N in yield (kg ha^{-1}) removed from field. The calculations of NRE and NB assume equal mineralisation of soil N on all plots.

Statistical analysis

Analysis of variance (ANOVA) by general linear model (GLM) in Minitab 17 (Minitab Inc, State College, PA, USA) was performed for yield, N and quality variables. For each location separately, we used a model with fertiliser treatment as a fixed factor, while year, interaction between fertiliser treatment and year, and replication nested within year was used as random factors. To enable the use of Tukey's multiple comparison test on treatment differences ($P = 0.05$) in Minitab, all factors were considered fixed.

Regression analysis was performed in Minitab 17 of yield, N and quality variables on potentially plant-available N from fertiliser materials during the growing season as estimated by Øvsthus et al. (2015) from results obtained by Øvsthus et al. (manuscript in preparation) during incubation of the fertilisers in soil at controlled temperature and moisture.

Results

Yield responses

All crops yielded well with shrimp shell (SS), anaerobically digested food waste (AD) and mineral fertiliser (MF) (Tables 4, 5). With algae meal (AM), however, the yields and N uptake tended to be smaller than with no fertiliser (NF), but the difference was not statistically significant. The yields with sheep manure (SM) were intermediate.

Broccoli yield has previously been presented by Øvsthus et al. (2015). In brief, on the average across 2 years and two locations, application of 170 kg N ha^{-1} as MF, AD, SS and SM resulted in, respectively, 106, 68, 55 and 32% larger yield than with NF, whereas AM fertilisation gave 53% smaller yield. Yields after AD and MF fertilisation (170 kg N ha^{-1}) were not significantly different across year and location (data not shown). A similar yield pattern was observed for broccoli fertilised with 80 kg N ha^{-1}, but the differences between treatments were smaller.

Potato and lettuce fertilised with 80 and 60 kg N ha^{-1}, respectively, showed a similar yield pattern as for broccoli (Tables 4, 5). Fertilisation with MF, AD and SS, respectively, resulted on the average across 2 years and two locations in 55, 31, and 42% larger potato yield than NF. The corresponding figures for lettuce were 76, 34 and 43%. Yields obtained with SS and MF fertilisation for potato (80 kg N ha^{-1}) and lettuce (60 kg N ha^{-1}) were not significantly different across year and location (data not shown).

Yields of broccoli, potato and lettuce were linearly correlated to our estimated amount of potentially plant-available N from the fertilisers during the growing season of the test crops (results not shown in figures or tables). Regression analysis conducted over year and location resulted in R^2 values of 50.5, 14.2 and 48.6 ($p < 0.001$), respectively, for broccoli, potato and lettuce. Year and location effects occurred for yields of broccoli and potato in 2009 and 2010.

Size, quality and marketable yield

Generally, the broccoli quality was marketable, with first class quality as described in NS2823:1999, except some occurrence of uneven maturity of buds within heads, heads with buds that did not mature and some small heads (below 60 mm diameter). Broccoli fertilised with AM had a high percentage that did not meet first-class size requirement and a high percentage of heads not harvested. Broccoli fertilised with MF, AD and SS at high N level (170 kg N ha^{-1}) tended to have a larger proportion of broccoli > 100 mm (Fig. 1).

Potato size distribution tended to be the same with all fertilisers except for AM, which had a higher proportion of larger-sized tubers (Fig. 1). This result was found both in the year when AM was applied at a rate of 80 kg N ha^{-1} and when the residual effect of previous AM application was determined. In the growing season, the tallest potato haulm was observed

Table 4 Yield and selected quality parameters on the Grimstad site for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF)

Treatment code*	Broccoli				Potato					Lettuce		
	Total yield (kg ha^{-1})	Mean head wt (g pl^{-1})	Size-discarded (% < 60 mm)	Head harvested (% of planted)	Total yield (kg ha^{-1})	Mean tuber wt. (kg pl^{-1})	Physical damage (%)	Size-discarded (% < 40 mm)	Mean haulm ht. (mm)	Total yield (kg ha^{-1})	Mean head wt (g pl^{-1})	Discarded (%)
AD2	11,338^{ab}	341.0^{ab}	0^{b}	86.7^{a}	16,116^{c}	0.4255^{c}	10.6^{ab}	24.3	576.1^{c}	34,966^{abcd}	559.5^{abcd}	0
SS2	9612^{bc}	315.2^{ab}	0.5^{b}	83.2^{ab}	16,869^{c}	0.4453^{c}	7.6^{ab}	18.7	583.0^{c}	35,946^{abc}	575.1^{abc}	0
SM2	9511^{bc}	285.3^{bc}	0^{b}	86.8^{a}	20,047^{bc}	0.5292^{bc}	3.1^{b}	17.0	623.1^{bc}	37,648^{ab}	602.4^{ab}	0
AM2	3267^{e}	159.5^{e}	7.2^{ab}	50.5^{c}	20,728^{abc}	0.5472^{abc}	11.7^{ab}	14.0	644.0^{bc}	20,512^{e}	328.2^{e}	33.4
AD1	9471^{bc}	267.0^{bc}	0^{b}	92.0^{a}	20,802^{abc}	0.5492^{abc}	3.0^{b}	25.6	707.6^{ab}	25,817^{de}	413.1^{de}	22.2
SS1	8899^{bc}	253.1^{bcd}	0.3^{b}	92.2^{a}	22,956^{ab}	0.6061^{ab}	8.1^{ab}	15.8	690.2^{b}	27,792^{bcde}	444.7^{bcde}	21.1
SM1	9456^{bc}	286.4^{bc}	3.2^{ab}	91.3^{a}	20,589^{abc}	0.5435^{abc}	4.9^{b}	20.3	689.1^{b}	33,104^{abcd}	529.7^{abcd}	2.5
AM1	4641de	165.9^{de}	13.0^{a}	67.3^{bc}	17,075^{c}	0.4508^{c}	21.3^{a}	17.0	627.0^{bc}	35,458^{abcd}	567.3^{abcd}	5.8
MF	13,915^{a}	379.0^{a}	0^{b}	94.0^{a}	25,843^{a}	0.6823^{a}	3.6^{b}	16.2	807.1^{a}	40,878^{a}	654.1^{a}	0
NF	7267^{cd}	208.1^{cde}	0.3^{b}	91.3^{a}	15,774^{c}	0.4164^{c}	10.4^{ab}	20.2	559.0^{c}	27,436^{cde}	439.0^{cde}	27.4
Mean values across treatments within year												
2009	10,188^{a}	281.9^{a}	3.6	91.8^{a}	18,775^{b}	0.4957^{b}	4.62^{b}	17.7	660.2			
2010	7288^{b}	250.2^{b}	1.3	75.3^{b}	20,585^{a}	0.5435^{a}	12.20^{a}	20.2	641.1	31,956	511.3	11.2
P values from ANOVA												
T	0.000	0.000	0.008	0.000	0.000	0.000	0.008	NS	0.000	0.000	0.000	NS
Y	0.000	0.012	NS	0.000	0.018	0.18	0.001	NS	NS			
T × Y	NS	NS	NS	0.006	0.032	0.032	NS	NS	NS			
Replication (Y)	NS	NS	0.049	NS	0.009	0.009	0.011	0.000	0.004	0.026	0.026	0.042

For detailed explanation of treatments and measured parameters, see the text and Table 2). For broccoli and potato, results are means of data from 2009 to 2010, and for lettuce, results are from 2010 only. Different letters within a column denote statistically significant difference at $P < 0.05$ according to Tukey's range test, and the p values pertain to effects of treatment (T), year (Y) and replication nested within year [Replication(Y)] as determined in ANOVA

Total fresh weight yield, mean fresh weight (wt) per plant (head or tuber), % discarded due to incorrect size (including quality disorder for lettuce), broccoli head harvested (% of planted), tubers with physical damage (% of total yield with errors due to green tuber, hollow heart and crack growth) and average potato haulm height (ht.)

* Treatment codes according to Table 2

Table 5 Yield and selected quality parameters* on the Bodø site for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF)

Treatment code*	Broccoli				Potato					Lettuce			
	Total yield (kg ha^{-1})	Mean head wt. (g pl^{-1})	Size-discarded (% ≤ 60 mm)	Head harvested (% of planted)	Total yield (kg ha^{-1})	Mean tuber wt. (kg pl^{-1})	Physical damage (%)**	Size-discarded (% < 40 mm)	Mean haulm ht. (mm)	Total yield (kg ha^{-1})	Mean head wt. (kg pl^{-1})	Size-discarded (% < 350 g)	Discarded (%)
AD2	8337^{ab}	243.9^{ab}	1.0^{a}	78.2^{ab}	31,974^{bcde}	0.7386^{bcde}	6.41^{ab}	18.89^{a}	546.6^{c}	47,820^{bc}	0.5356^{bc}	12.92^{bc}	5.4^{ab}
SS2	8585^{ab}	223.8^{ab}	6.2^{a}	86.5^{a}	30,181^{cde}	0.6972^{cde}	9.84^{ab}	19.52^{a}	520.8 ^{cd}	52,363^{ab}	0.5865^{ab}	4.79^{c}	2.6^{a}
SM2	6013^{bc}	182.3^{bcd}	5.5^{a}	77.2^{ab}	26,551^{e}	0.6133^{e}	8.94^{ab}	15.01^{ab}	491.0^{cde}	36,436^{bcd}	0.4081^{bcd}	42.41^{abc}	26.9^{abc}
AM2	2192^{d}	90.2^{e}	12.7^{a}	54.7^{c}	27,940^{de}	0.6454d^{e}	4.74^{ab}	12.88^{ab}	452.4^{de}	28,242^{d}	0.3163d	68.31^{a}	100.0^{d}
AD1	7889^{ab}	215.8^{abc}	10.5^{a}	84.7^{a}	36,224^{abc}	0.8368^{abc}	2.20^{b}	19.16^{a}	640.0^{ab}	38,392^{bcd}	0.4300^{bcd}	31.35^{abc}	31.3^{abc}
SS1	6548^{bc}	192.4^{bcd}	4.3^{a}	78.8^{ab}	39,049^{ab}	0.9020^{ab}	6.87^{ab}	17.60^{ab}	655.0^{a}	39,422^{bcd}	0.4415^{bcd}	36.71^{abc}	36.7^{abc}
SM1	4797 ^{cd}	152.2^{cde}	8.5^{a}	73.0^{abc}	34,533^{bcd}	0.7977^{bcd}	8.79^{ab}	14.02^{ab}	556.5^{bc}	32,589 ^{cd}	0.3650 ^{cd}	47.61^{ab}	47.6^{bc}
AM1	3018^{d}	102.2^{e}	12.7^{a}	64.8^{bc}	27,040^{e}	0.6246^{e}	17.76^{a}	7.65^{b}	421.2^{e}	44,614^{bcd}	0.4997^{bcd}	30.42^{abc}	30.4^{abc}
MF	10225^{a}	284.8^{a}	0.8^{a}	83.2^{ab}	41,646^{a}	0.9620^{a}	5.73^{ab}	16.22^{ab}	660.1^{a}	67,821^{a}	0.7596^{a}	0.00^{c}	0^{a}
NF	4481 ^{cd}	132.1^{de}	11.3^{a}	78.3^{ab}	27,918^{b}	0.6449^{b}	15.07^{ab}	18.40^{a}	493.9^{cde}	34,467^{bcd}	0.3860^{bcd}	50.05^{ab}	50.0^{c}
Mean values across treatments within year													
2009	7075.8^{a}	186.1	10.0^{a}	85.3^{a}	40,042^{a}	0.9250^{a}	10.84^{a}	8.61^{b}	627.8^{a}				
2010	5342.0^{b}	177.9	4.7^{b}	66.5^{b}	24,570^{b}	0.5676^{b}	6.43b	23.26^{a}	459.7^{b}	42,217	0.4728	32.46	33.1
P values from ANOVA													
T	0.000	0.000	0.035	0.000	0.000	0.000	0.018	0.017	0.000	0.000	0.000	0.000	0.000
Y	0.000	NS	0.006	0.000	0.000	0.000	0.021	0.000	0.000				
T × Y	NS	NS	NS	NS	0.001	0.001	NS	NS	0.003				
Replication (Y)	0.001	0.018	0.006	0.010	NS	NS	0.000	NS	NS	0.000	0.000	0.001	0.004

For detailed explanation of treatments and measured parameters, see the text and Table 2. For broccoli and potato, results are means of data from 2009 to 2010, and for lettuce, results are from 2010 only. Different letters within a column denote statistically significant difference at $P < 0.05$ according to Tukey's range test, and the p values pertain to effects of treatment (T), year (Y) and replication nested within year [Replication(Y)] as determined in ANOVA

* Total fresh weight yield, mean fresh weight (wt) per plant (head or tuber), % discarded due to incorrect size (including quality disorder for lettuce), broccoli head harvested (% of planted), tubers with physical damage (% of total yield with errors due to green tuber, hollow heart and crack growth) and average potato haulm height (ht.)

** Treatment codes according to Table 2

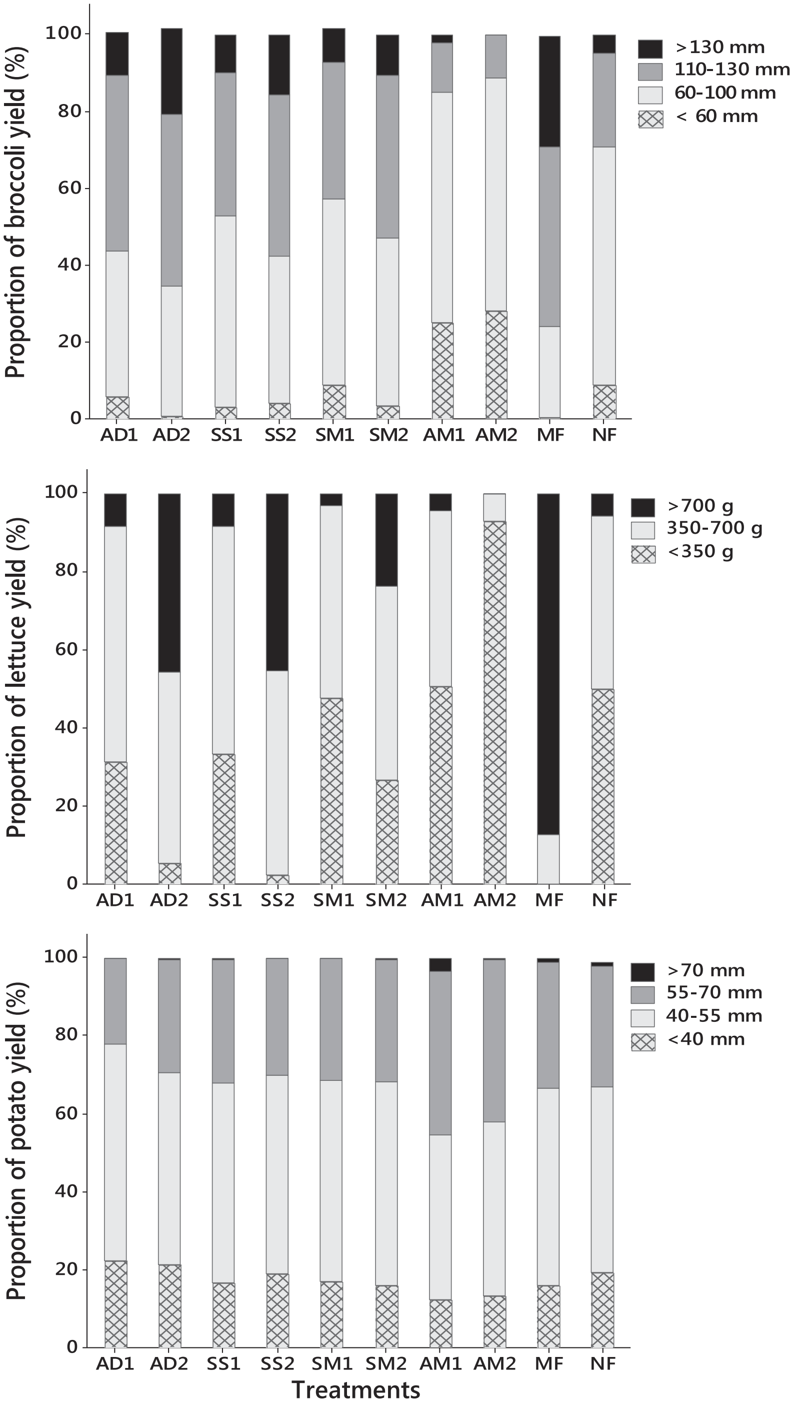

Fig. 1 Size distribution for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF). For detailed explanation of treatments and measured parameters, see the text and Table 2. Results are means of two locations (Bodø and Grimstad) and of 2 years for broccoli and potato and values for 1 year and one location (Bodø) for lettuce

with MF, AD and SS (Tables 4, 5). The percentage tubers with physical damage was highest with AM fertilisation, however, the difference was only significant when GLM analysis was conducted for results across both years and locations.

Lettuce treated with MF, SS and AD had clearly larger heads than lettuce fertilised with AM and NF (Fig. 1), resulting in a large proportion of heads meeting the first-class size limit of 350 g. With AM, more than 90% of the total yield did not meet the first-class quality standards. Lettuce fertilised with MF obtained higher NO_3^- content than with the other fertilisers at 60 kg N ha^{-1}, but it was not significantly different from that of AD-fertilised lettuce. The content of NO_3^- in lettuce ranged on the average across locations in year 2010 from 6.1 to 157.3 mg kg^{-1} fresh weight (AD1 Grimstad and MF Bodø, respectively; data not shown).

N uptake, N content and N balance

For all crops, total N uptake was smallest on NF and AM plots, and largest in MF-fertilised broccoli and lettuce (Tables 6, 7). For potato, the N uptake was similar for MF, AD and SS. The average N uptake values across year and location were in the range of 63.5–165.1, 40.8–96.3, and 20.6–65.7 kg N ha^{-1} in broccoli, potato and lettuce, respectively. For all crops in both years and on both locations, the N uptake was positively correlated with estimated potentially plant-available N from the organic fertiliser materials (Fig. 2).

The treatment effects on plant N content were small (Tables 6, 7). The average values across year and location were in the range of 16–33, 11–12 and 13–32 g kg^{-1} in broccoli, potato and lettuce, respectively. In broccoli and lettuce, the N contents were highest with MF and AD. The results for potato, however, did not show a similar pattern.

The N balance of the 3-year cropping sequence was positive for all treatments except for NF (Tables 6, 7). The ranking of N balance of the treatments in increasing order was NF < MF < AD < SS < SM < AM.

Apparent N recovery efficiency

NRE was affected by fertiliser treatment (Fig. 3), and on the average across year and location the values ranged from −9 to 57, −13 to 56 and −20 to 65% for broccoli, potato and lettuce, respectively. AM resulted in negative NRE, which was positively correlated with potentially plant-available N ($R^2 = 35.5$, 55.6 and 40.7 for broccoli, potato and lettuce, respectively; $P = 0{,}000$). In all crops, highest NRE was found with MF fertilisation, but it was not significantly higher than NRE obtained by SS2 (shrimp shell at 170 kg N ha^{-1}) and AD1 (anaerobically digested food waste at 80 kg N ha^{-1}) in broccoli, and SS1 (shrimp shell at 80 kg N ha^{-1}) and AD1 in potato. NRE obtained with SM (sheep manure) was intermediate.

Mineral N in soil and residual effects

After the harvest of broccoli in autumn, there were differences in content of inorganic N in plots at the upper N level of AD (AD2) compared to plots fertilised with other organic materials. The difference was found both in the upper and lower soil layers. The difference was not significantly different form MF-fertilised plots. Contents of inorganic N in soil after growing potato or lettuce were not affected by fertiliser treatments. The residual effect of fertilisation in previous years on yield of unfertilised potato and lettuce was small or undetectable. The content of inorganic N in soil in spring was not significantly influenced by the fertilisation treatments in previous years (data not shown).

Discussion

There were positive linear relationships between yield, N uptake, NRE or tested quality parameters, and the estimated potentially plant-available N from the fertiliser materials, which was inversely correlated with C:N ratio of the different materials (Øvsthus et al., manuscript in preparation). This is in agreement with a normally strong yield-limiting effect of sub-optimal N availability (Cassman et al. 2002; Zebarth et al. 1995), as typically found in organic agriculture (Berry et al. 2002), and with the relatively high negative correlation usually found between N mineralisation and the C:N ratio of organic materials (e.g., Nicolardot et al. 2001). Yield, N uptake and NRE depend on a complex range of factors including those affecting N mineralisation, N losses and crop N demand (Mosier et al. 2004). Therefore, deviations from linear relationships and for deviant single observations are to be expected.

Table 6 Nitrogen content, total N uptake, harvested N and N balance (accumulated N input and output in the cropping system) on the Grimstad site for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF)

Treatment code	Broccoli				Potato				Lettuce		
	N content (g kg^{-})	Total N uptake (kg N ha^{-1})	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})	N content (g kg^{-1})**	Total N uptake (kg N ha^{-1})**	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})	N content (g kg^{-1})	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})
AD2	26.4^{a}	139.9^{ab}	72.6^{ab}	97.4	11.5^{ab}	50.1^{bc}	29.9^{c}	67.5	19.7^{ab}	31.8^{ab}	95.7
SS2	23.8^{abd}	145.2^{ab}	64.8^{abc}	105.2	11.3^{ab}	48.8^{bc}	32.0^{c}	73.2	18.2^{bcd}	32.2^{ab}	101.0
SM2	21.4^{bc}	115.9^{bc}	56.2^{bcd}	113.8	11.3^{ab}	62.7^{bc}	37.1^{bc}	76.7	18.8^{abc}	32.1^{ab}	104.6
AM2	15.8^{d}	70.5^{d}	28.8^{e}	141.2	10.1^{b}	46.6^{bc}	37.5^{bc}	103.7	13.4^{e}	13.3^{c}	150.4
AD1	22.0^{bc}	109.2^{bc}	52.0 ^{cd}	28.0	11.6^{ab}	64.4^{bc}	45.0^{ab}	63.0	13.6^{de}	20.4^{bc}	42.6
SS1	20.9^{c}	113.3^{bc}	50.6 ^{cd}	29.4	11.5^{ab}	73.2^{ab}	46.3^{ab}	63.1	14.3^{cde}	23.1^{bc}	40.0
SM1	22.1^{bc}	112.1^{bc}	54.7 ^{cd}	25.3	12.4^{ab}	69.6^{ab}	41.9^{b}	63.4	14.1^{de}	24.9^{bc}	38.5
AM1	16.1^{d}	69.0^{d}	28.5^{e}	51.5	12.9^{a}	40.4^{c}	31.3^{c}	100.2	14.3^{cde}	26.4^{b}	73.8
MF	25.4^{ab}	174.4^{a}	79.9^{a}	91.1	12.6^{ab}	92.6^{a}	54.0^{a}	117.1	23.1^{a}	42.0^{a}	135.1
NF	19.8^{cd}	82.0^{cd}	40.6^{de}	−40.6	10.9^{ab}	50.5^{bc}	30.3^{c}	−70.9	14.5^{cde}	22.9^{bc}	−93.8
Mean values across treatments within year											
2009	25.8^{a}	133.3^{a}	65.5^{a}		ND	ND	41.1^{a}		ND	ND	
2010	17.0^{b}	93.0^{b}	40.2^{b}		11.6	59.9	35.9^{b}		16.4	26.9	
P values from ANOVA											
T	0.000	0.000	0.000		0.043	0.000	0.000		0.000	0.000	
Y	0.000	0.000	0.000				0.000				
T × Y	NS	NS	NS				0.004				
Replication (Y)	NS	NS	NS		0.034	0.000	0.000		NS	NS	

For detailed explanation of treatments and measured parameters, see the text and Table 2. For broccoli and potato, results are means of data from 2009 and 2010, and for lettuce, results are from 2010 only. Different letters within a column denote statistically significant difference at $P < 0.05$ according to Tukey's range test, and the p values pertain to effects of treatment (T), year (Y) and replication nested within year [Replication(Y)] as determined in ANOVA

* Treatment codes according to Table 2

** Results from year 2010 only

Table 7 Nitrogen content, total N uptake, harvested N and N balance (accumulated N input and output in the cropping system) on the Bodø site for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF)

Treatment code	Broccoli				Potato				Lettuce		
	N content (g kg^{-1})	Total N uptake (kg N ha^{-1})	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})	N content (g kg^{-1})	Total N uptake (kg N ha^{-1})	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})	N content (g kg^{1})	N in harvested part (kg N ha^{-1})	N balance (kg N ha^{-1})
AD2	32.6^{a}	133.9^{ab}	51.7^{ab}	118.3	10.7^{a}	89.11^{bc}	64.0^{bc}	54.3	29.0^{ab}	57.0^{ab}	57.3
SS2	29.5^{ab}	131.0^{abc}	44.7^{abc}	125.3	10.9^{a}	83.16^{c}	60.1^{c}	65.2	26.7^{bc}	59.8^{ab}	65.4
SM2	26.1^{bc}	97.1^{cde}	36.7^{bcd}	133.3	10.5^{a}	71.81^{c}	50.4^{c}	82.9	23.8^{c}	42.4^{bc}	100.5
AM2	18.2^{d}	56.5^{f}	18.8^{e}	151.2	11.5^{a}	78.21^{c}	57.0^{c}	94.2	25.0^{bc}	24.7^{c}	129.5
AD1	28.4^{ab}	111.8^{bcd}	42.2^{abcd}	37.8	12.3^{a}	119.19^{ab}	85.7^{ab}	32.1	26.9^{abc}	51.7^{abc}	–19.6
SS1	27.8^{ab}	110.8^{bcd}	39.3^{abcd}	40.7	11.4^{a}	117.12^{ab}	84.3^{ab}	54.5	26.2^{bc}	51.9^{abc}	2.6
SM1	24.9^{bc}	84.0^{def}	30.5^{cde}	49.5	10.6^{a}	91.99^{bc}	66.2^{bc}	63.3	26.0^{bc}	44.2^{bc}	19.1
AM1	22.2^{cd}	70.3^{ef}	20.4^{e}	59.6	12.1^{a}	66.22^{c}	48.1^{c}	91.5	26.0^{bc}	54.1^{ab}	37.4
MF	32.8^{a}	155.7^{a}	54.7^{a}	115.3	12.2^{a}	127.78^{a}	94.4^{a}	100.9	31.5^{a}	78.9^{a}	82.0
NF	24.9^{bc}	73.4^{ef}	$27.0d^{e}$	−27.0	10.7^{a}	79.51^{c}	57.0^{c}	–84	26.3^{bc}	45.5^{bc}	−129.5
Mean values across treatments within year											
2009	24.8^{b}	130.0^{a}	44.0^{a}		11.6^{a}	116.44^{a}	79.8^{a}				
2010	28.7^{a}	74.9^{b}	29.2^{b}		10.9^{b}	68.38^{b}	53.6^{b}		26.7	51.0	
P values from ANOVA											
T	0.000	0.000	0.000		0.003	0.000	0.000		0.001	0.001	
Y	0.000	0.000	0.000		0.005	0.000	0.000				
T × Y	NS	NS	NS		0.019	NS	NS				
Replication (Y)	0.044	NS	0.001		0.044	NS	NS		NS	0.004	

For detailed explanation of treatments and measured parameters, see the text and Table 2. For broccoli and potato, results are means of data from 2009 to 2010, and for lettuce, results are from 2010 only. Different letters within a column denote statistically significant difference at $P < 0.05$ according to Tukey's range test, and the *p* values pertain to effects of treatment (T), year (Y) and replication nested within year [Replication(Y)] as determined in ANOVA

* Treatment codes according to Table 2

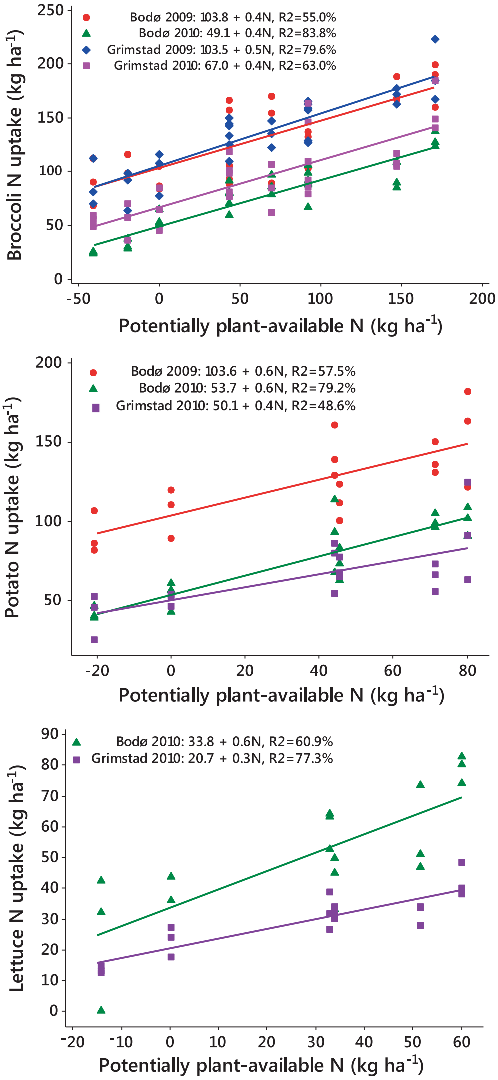

Fig. 2 Measured N uptake in broccoli, potato and lettuce (dots) as a linear function (lines) of potentially plant-available N during the growing season as estimated by Øvsthus et al. (2015) from results obtained by Øvsthus et al. (manuscript in preparation) during incubation of the fertilisers in soil at controlled temperature and moisture. Results are means for each location and year

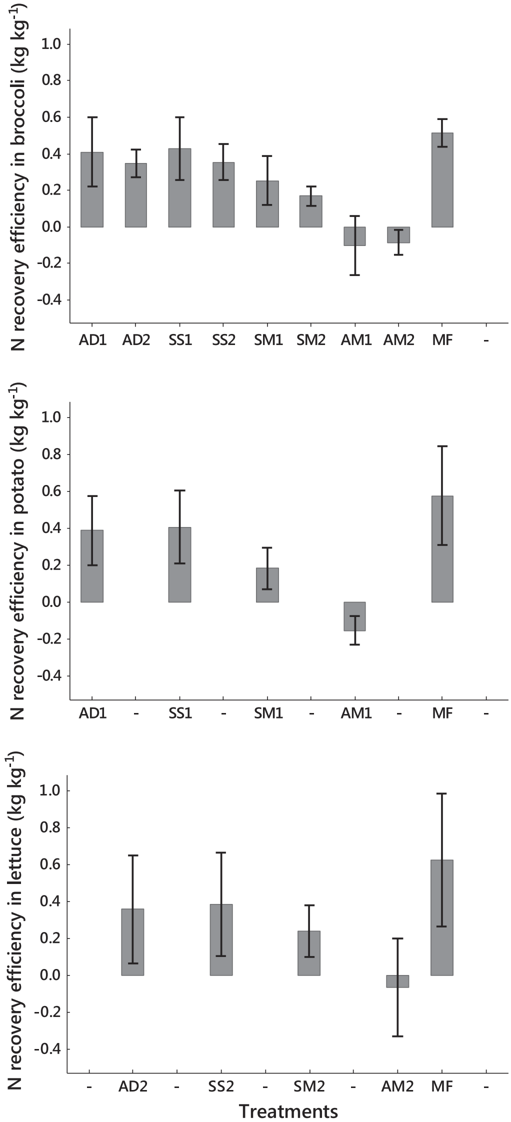

Fig. 3 Recovery efficiency of applied N (NRE = $(U-U_0)/N_A$) for broccoli, potato and lettuce in a 3-year cropping sequence with anaerobically digested food waste (AD), shrimp shell (SS), sheep manure (SM) and algae meal (AM) as fertilisers at two N application rates (1 and 2), mineral fertiliser (MF) and no fertiliser (NF). For detailed explanation of treatments and measured parameters, see the text and Table 2. Results are means of two locations (Bodø and Grimstad) and of 2 years for broccoli and potato and values for 1 year for lettuce. The bars show 95% confidence intervals of the mean

The results for AM, i.e., the lowest yield, N uptake and NRE and the highest N balance values, were remarkable to the extent that this dried and milled seaweed product is being marketed as fertiliser and soil conditioner (http://www.algea.com/index.php/algeafert-meal). However, the results were expected considering its relatively high C:N ratio (C:N = 37) and net immobilisation detected in the incubation experiment by Øvsthus et al. (manuscript in preparation) and are in accordance with results of other studies on materials with similar decomposability and C:N ratios (Breland 1996a, b; Jensen et al. 1999; Vigil and Kissel 1991). Breland (1996a, b) found that ryegrass with a C:N ratio of 26–50 (depending on plant part and N fertilisation), in incubation tended to cause a small temporary net N immobilisation and a tendency of only a very limited re-mineralisation during a time period comparable to the present experiment. In the present experiment with AM, there was neither higher concentration of NO_3^- in soil in autumn or subsequent spring nor larger yield recorded as residual effect of AM fertilisation. This is consistent with the finding of Breland (1996b) that a ryegrass crop ploughed into soil in late autumn had a close to neutral residual effect on subsequent spring grain. Nevertheless, a positive effect on soil N mineralisation may be expected after several years of AM application due to accumulated immobilisation of N, the size of which eventually will become large enough to contribute significantly to crop N supply by its re-mineralisation, in spite of small contributions from each single-year cohort. For example, in a crop rotation experiment, Breland and Eltun (1999) observed increased C and N mineralisation rates for an extended period of incubation (449 days at 15 °C) in soil that for only 5 years had received more organic matter as perennial root growth, plant residues and animal manure, as compared to an all-arable cropping sequence without animal manure. Their results could be modelled as mainly an increase in two conceptual pools of soil organic matter with carbon half-lives at 15 °C of 0.76 and 12.7 years, respectively. Consequently, the present results, in agreement with previous ones (Asdal and Breland 2003; Breland 1996a, b; Jensen et al. 1999; Vigil and Kissel 1991), suggest that when there is a need for a relatively rapid and predictable N supply for N-demanding crops such as broccoli, materials with a high concentration of inorganic N such as AD, or a rapidly net N mineralising material such as SS should be used. The short-term effects of SM in the present experiment were intermediate, most likely due to relatively stable C compounds (Asdal and Breland 2003). A low C:N ratio and a high concentration of inorganic N at the time of application for materials such as AD and SS could be combined with materials of higher C:N ratio, such as AM, in order to build up a more stable long-term soil N mineralisation capacity and to reduce the likelihood of ammonia volatilisation, nitrous oxide emission and nitrate leaching shortly after application.

Little is still known about decomposition and N mineralisation from algae. However, it seems likely that species with lower C:N ratio than the current AM will give a more positive short-term net N mineralisation (Jensen et al. 2005; Nicolardot et al. 2001) and, consequently, fertiliser effect on N-demanding crops.

In addition to neutral or negative net N mineralisation from AM, other factors might have contributed to its poor effects on crop yields. AM has a total S content five times higher than that of MF. However, plants are generally not sensitive to high S level in soils (Mengel and Kirkby 2001). Salt concentration in the fertilisers was not measured, but NaCl in seaweeds may have influenced yield. Typical Na^+ and Cl^- toxicity symptoms were not seen, although yellowish leaves were observed. However, these symptoms could equally well have been caused by deficiency of N, as suggested by the negative net N mineralisation from AM (data not shown). As both lettuce and potato are sensitive to Cl^- toxicity, further research is needed to determine whether NaCl concentrations in seaweed products are sufficiently low to avoid toxic effects on plant growth.

SS and AD had fertiliser effects that did not differ significantly from those of MF. The NRE for all MF-treated crops were more than 50%, which is similar to results for broccoli reported by Zebarth et al. (1995), but lower than found by Vågen (2005). Quality of fertiliser material, timing and amount of plant-available N, the type of mineral N (NH_4^+ or NO_3^-), N immobilisation, ammonia volatilisation, nitrous oxide emission and nitrate leaching may potentially explain some of the gap between applied N and apparent N recovery in crops (Cameron et al. 2013; Galloway et al. 2003; Raun and Johnson 1999). In addition to the yield and N data, the crop quality indices measured in the field experiments (discarded product, damages (physical or disease), per cent harvested, N content, height of potato haulm, size distribution) also suggested that the effects of AD and SS were similar to those of MF. The high proportion of damage and discarding by AM fertilisation is in accordance with other fertiliser experiments that have included treatments that gave similar N availability (Doltra et al. 2011).

The higher NO_3^- concentration in lettuce fertilised with MF compared to other treatments could be explained by the amount, availability of N and form of mineral N at application, which is found in other experiments as well (Anjana et al. 2007; Chena et al. 2004; Santamaria et al. 2001). Due to reduced N availability, vegetables fertilised with organic materials often are lower in NO_3^- concentration than vegetables having received inorganic fertiliser at similar N rates (Raupp 1996). If N is present as NH_4^+, as in AD and SM, the level of NO_3^- in vegetables has been found to be lower than when N is in the form of NO_3^- (Santamaria et al. 2001), which can accumulate in crops and be stored in the vacuole. In the current experiment, the fertilisers were supplied prior to planting and the total N supply was small, and all NO_3^- concentrations were low compared to studies performed by Santamaria (2006).

Conclusions

1. Fertiliser effects on yield, N uptake, NRE, N balance and quality parameters of vegetable crops were to a large extent explained by the potential amount of inorganic N becoming available during the growing season, as estimated on the basis of results obtained by Øvsthus et al. (manuscript in preparation) during incubation of the fertilisers in soil at controlled temperature and moisture. Consequently, such a test seems essential for selecting alternative fertilisers, deciding on application rates and predicting effects on crop yield and quality.
2. The materials with the most inorganic N at application or large net N mineralisation had fertiliser effects similar to those of mineral fertiliser, showing a potential for turning waste or unutilised materials into resources with the potential for replacing mineral N fertilisers.
3. No residual effect was detected in the year after application, but the materials with weaker or no fertiliser effect and less or no net N mineralisation may, if used repeatedly, be expected to contribute to the more long-term capacity of soil to provide plant-available N.
4. To supply adequate fertiliser for N-demanding crops in the short term while also increasing the more long-term N-supplying capacity of the soil, it seems desirable to combine the use of waste or alternative fertiliser materials that release plant-available N rapidly with materials retaining or causing immobilisation of N. To judge whether such materials should be mixed or kept separate in time or space requires further investigation.

Acknowledgements We are grateful to the Research Council of Norway, the counties of Nordland and Troms, and the Norwegian Institute of Bioeconomy Research for financial support of this work.

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Paper III

Øvsthus I, Thorup-Kristensen K, Seljåsen R., Riley H, Dörsch P and Breland TA, 2021. Calibration of the EU-Rotate_N model with measured C and N mineralization from potential fertilizers and evaluation of its prediction of crop and soil data from a vegetable field trial. European Journal of Agronomy, in review; revised and resubmitted.

Calibration of the EU-Rotate_N model with measured C and N mineralization from potential fertilizers and evaluation of its prediction of crop and soil data from a vegetable field trial

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ABSTRACT

Mechanistic models are useful tools for understanding and taking account of the complex, dynamic processes such as carbon (C) and nitrogen (N) turnover in soil and crop growth. In this study, the EU-Rotate_N model was first calibrated with measured C and N mineralization from nine potential fertilizer resources decomposing at controlled soil temperature and moisture. The materials included seaweeds, wastes from the food industry, food waste anaerobically digested for biogas production, and animal manure. Then the model's ability to predict soil and crop data in a field trial with broccoli and potato was evaluated. Except for seaweed, up to 68% of added C and 54–86% of added N was mineralized within 60 days under controlled conditions. The organic resources fell into three groups: seaweed, high-N industrial wastes, and materials with high initial content of mineral N. EU-Rotate_N was successfully calibrated for the materials of industrial origin, whereas seaweeds, anaerobically digested food waste and sheep manure were challenging. The model satisfactorily predicted dry matter (DM) and N contents (root mean square; RMSE: 0.11–0.32) of the above-ground part of broccoli fertilized with anaerobically digested food waste, shrimp shell pellets, sheep manure and mineral fertilizers but not algal meal. After adjusting critical %N for optimum growth, potato DM and N contents were also predicted quite well (RMSE 0.08–0.44). In conclusion, the model can be used as a learning and decision support tool when using organic materials as N fertilizer, but preferably in combination with other aids and information sources as, e.g., literature and field experiments.

Keywords: waste-derived organic fertilizers; recycling; carbon mineralization; nitrogen mineralization; broccoli; potato

1. INTRODUCTION

Recycling of organic materials is central to the circular bioeconomy, which is high on the political agenda in Norway and the EU (Meld.St. nr. 45 (2016-2017); COM 2015). In 2017, 99 300 Mg nitrogen (N) of mineral fertilizer was sold in Norway, and the corresponding amount for the EU was 11 600 000 Mg N (Eurostat 2017). Organic resources contain N and other nutrients of potential fertilizer value which could replace some of the mineral fertilizer used in agricultural and horticultural production. Using N from organic resources would be positive for both environment and production in several ways: Firstly, by reducing the enrichment of the biosphere with reactive N through the highly energy-demanding Haber-Bosch process (Galloway 2003); Secondly, by turning a waste problem into a positive resource; Thirdly, by contributing to carbon (C) storage in the soil and an increase in soil quality (Loveland and Webb 2003). Furthermore, local N sources are desirable for N-demanding vegetables, e.g., in organic cropping systems, as their use reduces the dependency on transportation of input factors.

The N fertilizer value of and N recovery from organic resources depend on how well the amount and dynamics of N mineralization from these materials match a crop's N demand. N mineralization depends on the quality of the added organic materials (AOM) and edaphic factors such as soil temperature and moisture, soil structure and texture, and soil pH. Properly calibrated and validated simulation models can help scientists and advisers to gain a better understanding of the complexity of processes involved during decomposition of organic materials and to predict effects of various factors on N mineralization, crop biomass and marketable yield when using organic materials as fertilizers.

Models for simulating C and N dynamics in soil differ in complexity regarding biogeochemical processes and spatial and temporal resolution. An important class of such models describe litter and soil organic matter as conceptual, homogeneous compartments decomposing at specific rates according to first-order kinetics. N mineralization is stoichiometrically linked to C mineralization from those compartments. Some of these models are included as modules of soil–plant ecosystem or soil–plant–atmosphere models designed to simulate plant growth and environmental impacts at field level (Manzoni and Porporato 2009).

The EU-Rotate_N model is a dynamic, deterministic soil–plant–atmosphere model developed primarily for vegetable crop rotations. The model takes account of C and N mineralization and soil organic matter dynamics, soil inorganic N, losses of N to the environment, water balance, root growth, crop growth, N uptake, marketable yield and economic return as influenced by environmental factors such as water, temperature, snow and frost and by agronomic practices, including fertilization (Rahn et al. 2010). The model is largely process-based but departs from its mechanistic orientation by introducing an empirical element when it comes to crop growth: "[….] a maximum achievable yield needs to be provided on the basis of the user's experience. This approach is considered the most feasible, considering the vast range of different crop types and morphologies among field vegetables and the resulting difficulties in applying generic photosynthesis-driven algorithms" (Nendel et al. 2013). The model has been calibrated for more than 70 vegetable and cereal species and has been tested in field studies in many parts of Europe (Rahn et al. 2010; Doltra and Munoz 2010; Nendel et al. 2013; Suarez-Rey et al. 2016) as well as in greenhouse studies (Guo et al. 2010; Sun et al. 2012; Soto et al. 2014). The calculation of N mineralization from organic matter in EU-Rotate_N is based on the routines used in the DAISY model (Hansen et al. 1991), which among available alternatives appears to be intermediately complex in terms of variables used to take account of microbial biomass, soil organic matter, mineralization products and the physical environment (Manzoni and Porporato 2009). The mineralization module of EU-

Rotate_N has been developed to simulate N release from soil organic matter and traditional organic fertilizers such as animal and green manures, but not from organic N resources such as industrial wastes and seaweed. Thus, the model has a potential to be further developed for locally available organic resources relevant for both organic and conventional vegetable production.

For a wide range of plant residues, there is data available on the dynamics of C and N mineralization (e.g., Jensen et al. 2005), examples of model calibration with (Henriksen and Breland 1999b) and testing against such data (Henriksen et al. 2007) and of testing under field conditions (Henriksen and Breland 1999a). To our knowledge, there are few studies—particularly with more comprehensive soil–crop–atmosphere models—on organic materials from the sea and recyclable wastes from the food industry, households and animal husbandry. Such studies are needed to understand how to include and make better use of these materials as fertilizers under various scenarios.

The aim of the present study was to calibrate the EU-Rotate_N model with C and N mineralization data from incubation of selected organic resources, and to evaluate the model performance by comparing subsequent predictions with results from a field experiment with broccoli (*Brassica oleracea*) and potato (*Solanum tuberosum*) conducted at Bodø in northern Norway. Our assumption was that waste-derived organic materials and algal meals may have decomposition patterns that differ from those of the crop residues, manure and slurries already included in the model and, therefore, require separate model calibration.

2. MATERIALS AND METHODS

2.1 Organic resources

In our experiment, we tested the following organic resources: 1) macro-algae (seaweeds) suitable for capturing nutrients in integrated multi-trophic aquaculture (IMTA; Wang et al. 2012; Marinho et al. 2015), *viz*., a commercial algal meal (AM), and washed, dried and ground algal meal of *Laminaria digitata* (LD) and *Saccharina latissima* (SL), 2) industrial waste with high N concentrations, *viz*., meat bone meal (MBM), shrimp shell powder (SSM), shrimp shell pellets (SSP) and dried fish sludge waste (FW), which was a combination of fish excrement and feed residues, 3) anaerobically digested food waste (AD) and 4) sheep manure (SM) including straw. The chemical composition of the nine waste-derived organic materials and macro-algae were analyzed by ALS Laboratory Group Norway AS, Oslo, Norway. Total Kjeldahl N (TKN) was determined according to ISO 937 and 1871 (TKN for SM was measured according to ISO 7150 -1,2/CSN 83 0530) and mineral N (NO_3^- and NH_4^+) by flow injection analysis according to local methods (SOP 8.18 A and SOP 8.64 A). The major chemical characteristics are shown in Table 1. MBM was produced by Norsk Protein AS, Mosvik, Norway. Similar MBM products have been described and tested by Jeng et al. (2004, 2006) and Brod et al. (2012, 2014). SSP and SSM were produced by Nofima, Bergen, Norway, and Bioprawns AS, Nord-Leangen, Norway, respectively. The production process of SSP is described in Johansen et al. (2019) and the material has been tested in pot and field experiments (Øvsthus et al. 2015, 2017; Johansen et al. 2019). FW is fish sludge waste which was collected from an on-land salmon hatchery, Åsen settefisk AS (Levanger, Norway). Similar products have been described by Brod et al. (2012, 2014, 2017). MBM, FW and SS are mainly composed of protein, fat and ash (Hendriks et al. 2002, Brod et al. 2018; Ibrahim et al. 1999). AD was digested household waste from the HRA biogas plant, using technology produced by BioTek AS. The product has been described and tested in several studies (Brod et al. 2017; Möller and Stinner 2009; Haraldsen et al. 2011). SM was from NIBIO Tjøtta, Norway. AM is a commercial product from Nordtang AS (Vestbygd, Norway), consisting mainly of the algae species *Ascophyllum nodosum*. SL and LD were collected from the shelf of the North Sea close to Bodø, washed, dried and ground. These macro-algae products are brown algae or seaweed, which vary in contents of protein and amino acids, carbohydrates and polysaccharides (alginate, sulphated fucose-containing polymer, fucoidan, cellulose, alginic acid, and lamarin), minerals, lipids and fiber (Øverland et al. 2018). Literature data on the compositions of the nine organic materials were used to estimate the initial values for pool fractions included in the model (see the paragraph about model calibration).

2.2 Incubation of organic materials in soil at controlled temperature and moisture

A dark brown sandy soil (orthic humo-ferric podzol, 1% coarse sand, 38% medium sand (0.6 – 0.2 mm), 52% fine sand (0.2 – 0.06 mm), 7% silt and 2% clay, pH in water 6.1, with 2.1% total carbon (TC) and 0.17 % total N (TN)) was sampled to 0.2 m depth at random positions from the field located at the former research farm Vågønes, Norwegian Institute for Agricultural and Environmental Research, Division Bodø, Norway, where the experiment was conducted. The field had been used as cattle pasture for more than 25 years. The soil was stored at ca. 4°C for 3 months in two black 50 L plastic pots covered with black plastic (not airtight). At the end of the storage period, the soil was air-dried at about 15°C, sifted (2 mm) and thoroughly mixed. A sample of 100 g soil was dried at 105˚C to determine its moisture content (dry weight; DW). Soil moisture of the samples to be incubated was then adjusted by addition of tap water to field capacity, which was determined previously by Haraldsen and Grønlund (1989) to be 30 % (i.e., drainable pore volume of 18% subtracted from total pore volume of 48%). Organic materials equivalent to 380 kg N ha^{-1} (when considering a 0.2 m plow layer; 0.007 g N 50 g DW $soil^{-1}$) were thoroughly mixed with 50 g DW soil and packed into 210 ml plastic cups (NorEngros AS, Norway). Unamended soil served as control. Each treatment, with or without incorporated organic materials, consisted of 15 samples, giving a total of 150 samples. The samples were placed in an incubator at day zero (Termaks B 8420S, Norway, Bergen) at 15°C for 60 days. A water tension, corresponding to 50% of field capacity at 5 kPa, was maintained by replenishing lost water to target weight twice a week. Triplicate cups were destructively sampled at days 1, 10, 18, 39 and 60 and frozen at –18°C for analysis of inorganic N (NH_4^+ and NO_3^-) at the Norwegian Institute of Bioeconomy Research (NIBIO, Apelsvoll Research Station, Kapp, Norway) where 40 g soil was extracted in 200 ml 1 M KCl and analyzed using a Flow Injection Analyser (FIAstar 5000, Foss Analytical AB, Sweden).

To determine C mineralization in the treatments, triplicate samples from each treatment were placed in sealed 2 L glass jars equipped with alkali traps for capturing evolved CO_2. The alkali traps consisted of 5 ml 1 M NaOH in 20 ml liquid scintillation vials. Amount and molarity of NaOH were calculated to ensure sufficient capacity for trapping evolving CO_2 throughout the closing intervals. The alkali traps were removed, sealed and replaced by fresh ones at day numbers 3, 7, 12, 19, 27, 38, 43 and 60. The C contents of the alkali solutions were analyzed at NMBU in an extraction line mixing Na_2CO_3 with 3 M H_2SO_4 in a closed mixing cell filled with glass beads, and extracting the evolving CO_2 in a stream of argon (Ar), which was flushed to an infrared gas analyzer (IRGA). Standard solutions of Na_2CO_3 dissolved in 1 M NaOH were used for internal calibration.

Carbon and nitrogen mineralization from the organic resources were estimated by subtracting CO_2-C evolved and mineral N accumulated in soils in unamended control soil from CO_2-C evolved and mineral N accumulated in soils amendment with fertilizer materials. The average of the three replicate control samples was subtracted from each of the three replicates with organic materials. Mineralization was expressed as percentages of added C or N, amounts of mineralized C or N (kg ha^{-1}) or as average C or N mineralization rates (kg ha^{-1} d^{-1}) within each time interval. As the C input data for the organic resources are not entered directly in the models input file, but are included indirectly by multiplying added DM by a constant factor of

0.45 (personal communication with Claas Nendel 4th of April 2019), and N input is calculated from C in each pool according to equation 2, the calibration was done in terms of C and N mineralization per hectare (Figure 4) instead of % of added C and N.

2.3 The mineralization module of EU-Rotate_N and its calibration

The mineralization module of EU-Rotate_N takes account of organic matter in three main pools: added organic matter (AOM), soil microbial biomass (SMB) and soil organic matter (SOM). Each pool is divided into two sub-pools with slow (AOMs, SMBs and SOMs) and fast (AOMf, SMBf and SOMf) decomposition rates, respectively. The decomposition follows first-order kinetics:

$$dC_x/dt = k_x C_x \qquad \text{(equation 1)}$$

where dC_x/dt is the turnover rate (kg C day^{-1}) of pool x (AOM, SMB or SOM pools), C_x is the content of carbon in pool x at time t and k is the first-order decomposition rate coefficient (decay rate constant, day^{-1}), which is fixed for each pool (Hansen et al., 1991). The decomposition rate constants are multiplied by rate-modifying coefficients, which are functions of soil temperature and moisture as estimated on a daily basis from weather data (driving variables). In the original version of EU-Rotate_N, C:N ratio and partitioning coefficient for the crop residue pools were derived from stepwise chemical digestion (Goering and Van Soest 1970) conducted by Jensen et al.(2005), whilst for manure and slurries the parameters were taken from the DAISY model. In organic materials where decomposition already has taken place, 10% of the C is not allocated to AOMs or AOMf.. The amounts of N in AOMs and AOMf are calculated from the amounts of C in the pools, in the official model version assuming a fixed C:N ratio for AOMs and that the remaining organic N resides in AOMf:

$N_t=C_t*N/C$ (equation 2)

where N_t is the amount of N in the actual pool at time t, C_t is the amount of C in the same pool at that time, and N/C is the reciprocal of C:N ratio in the respective pool. The daily loss of N from each pool is then proportional to the turnover of its organic C and the reciprocal of its C:N ratio.

In the present study, the initial C pools of the organic resources were first set by dividing total C into AOMs (slow pool) and AOMf (fast pool) according to model default values (Rahn et al. 2010). The proportions of these pools were, respectively, 38 and 62% in non-processed materials and 72 and 18% for processed materials. For some of the added materials, this resulted in poor fit with measured C mineralization. Therefore, estimation of the initial pool sizes for all the organic materials included in the model calibration was instead done *a priori* based on literature values on the biochemical quality of the AOM pools, which is hemicellulose and cellulose-like (AOMs pool) and soluble components (AOMf pool). It was difficult to find literature values for AM and SSP. Therefore, pool sizes for AM were set equal to those of LD and SL. Thus, for all brown algae, AOMs and AOMf were set at 65 and 35%, respectively. SSP pool sizes were set equal to those for SSM, due to its similar chemical composition, even though other fractionation alternatives resulted in a better shape of the curve and statistical indices for SSP. The partitioning of initial C is shown in Table 2.

The model calibration was then done by adjusting the values of the decomposition rate coefficients (*k* in equation 1 for fast and slow pools, respectively) and the C:N ratio of each pool (CN_slow and CN_fast) to obtain the best possible fit between simulated and measured values of C and N mineralization from the added resources. First, decomposition rate coefficients (*k*) for AOMs and AOMf of the materials were adjusted by trial and error until simulated C mineralization in the incubation experiment upon visual examination was considered to give the best possible representation of the measured values (both absolute level and shape of the time series). Four statistical indices were then used to possibly improve the match further (see section 2.6 below for details). Next, the C:N ratios of AOMs and AOMf for each organic material were adjusted to achieve the best possible fit, as judged both visually and statistically, between simulated and measured N mineralization. No fixed constraint was set on the range of the estimated parameter values, but values were kept within limits considered realistic based on data from relevant literature. The calibrated decay rate constants and C:N ratios for the AOMs and AOMf pools of each organic material are shown in Table 2.

By first setting initial AOMs and AOMf pool sizes according to literature values, then forcing the model to simulate measured C mineralization and finally N mineralization, equifinality due to simultaneous adjustment of sizes, decay rate constants and C:N ratio of each pool was ruled out. As decay rate constants of the two pools were adjusted simultaneously, there was some room for equifinality in simulation of C mineralization. It was limited, however, by the shapes of the mineralization curves. For most materials, the same is true for C:N ratios as estimated by fitting simulated values of N mineralization to those measured.

2.4 Model inputs for calibration and model performance evaluation

The model simulation period for the field experiment, which was conducted in 2008, 2009 and 2010, was from 1st January 2007 to 31st October 2010. The meteorological data were from a weather station located at Vågønes, Bodø, Norway, which is located nearby the field experiment. Air temperature (°C 2 m above ground), precipitation (mm), relative humidity (%), wind speed (m s^{-1} 2 m above ground), and global radiation (MJ m^{-2} d^{-1}) were included in the weather file. The model inputs include soil texture, bulk density, pH, organic matter, C:N ratio, water saturation, permanent wilting point and field capacity, initial soil moisture content and soil mineral N for three soil layers (0–0.3 m, 0.3–0.6 m and 0.6–0.9 m), and readily evaporable water values were measured in this experiment or taken from Haraldsen and Grønlund (1989). The model's runoff and snow–frost simulations were switched off. The set-up values are shown in Table 3. Further information entered in the input files on management, crop species, time of planting, date of harvesting and target DM yield, are listed in Table 4.

For the calibration of the N mineralization module, the weather input file was altered by setting fixed values of temperature to 15°C, rain to 0.1 mm (to avoid drying out of the soil), RH 80%, wind speed to 1 m s^{-1}, 2 h d^{-1} sunshine and global radiation 5 MJ m^{-2} d^{-1} (to ensure that model can be run).

Before running the model prediction of results from the field experiment, a target DM yield was set, which means that the highest achievable yield was estimated before running the model. According to Nendel et al. (2013), this approach is the best solution considering the vast variations of crop genetics, morphology and photosynthesis, which would otherwise require the use of very complex model algorithms. Target total DM yields were set at the highest total DM obtained with mineral fertilization at 80 and 170 kg N ha^{-1} for potato and broccoli, respectively (Table 4). The model then calculated daily crop growth as a function of day degrees, soil N status, temperature and soil moisture content.

The simulated crop growth is dependent on the crop-specific critical %N parameter, which is the lowest crop N concentration required for maximum growth during the growth period. This is expressed in relation to the total DM yield present at any time and is calculated as:

Critical %N=a(1+b*e–0.26W) (equation 3)

where W is total crop DM weight (Mg ha^{-1}) and a and b are crop-specific constants (Greenwood 1986). Originally, a and b for broccoli were 3.45 and 0.6, respectively, and 1.35 and 3 for potato. During the model evaluation, consistent underestimation was observed for potato yield and DM for all treatments including mineral fertilizer. Therefore, for potato the parameters of the equation 3 for critical %N was adjusted to fit the yield and DM for the mineral fertilizer treatment, resulting in a=0.70 and b=2.0.

The model has two strategies to calculate fresh yield: direct conversion or as single plant approach. The single plant approach is for plants with a single product per plant. The fresh-weight and DM yields are calculated by using the harvest index. Direct conversion is used for plants with multiple harvests or products per plant and is calculated multiply the total DM yield with a ratio to gain marketable fresh yield. The ratio is connected to the plant-available nitrogen. The predicted values presented here are those from the direct conversion approach (lower yield was found using the single plant approach).

2.5 Field experiment

The field experiment has been described in detail by Øvsthus et al. (2015, 2017). In short, a three-year factorial crop rotation experiment including broccoli (*Brassica Oleracea* L. *var. Italica* cv. Marathon; first-year crop), potato (*Solanum tuberosum* L. *cv. 'Troll'*; second-year crop) and lettuce (*Lactuca sativa* L. *cv. 'Ametist'* and *Lactuca sativa* L. *cv. 'Argentinas'*; third-year crop) was set up with three replicate blocks. Four organic fertilizer materials (Anaerobically digested food wastes (AD), Shrimp shell pellets (SSP), Sheep manure (SM) and Algal meal (AM)) were applied at rates equivalent to 80 and 170 kg N ha^{-1} for broccoli, 80 kg N ha^{-1} for potato and 60 kg N ha^{-1} for lettuce, and mixed into the soil. Plots with mineral fertilizer and no fertilizer served as control plots. More information about fertilization, management and cropping dates is given by Øvsthus et al. (2015, 2017) and Table 4.

In the first year of the field experiment, broccoli was planted on biodegradable film based on corn starch (BioAgri, BioBag Norge AS, Askim, Norway) with the aim of reducing leaching and weed growth. Due to problems with dissolution and mineralization of fertilizers in the upper soil layers close to the biofilm, this practice was abandoned in the following years. Thus, the results for broccoli in 2008 were omitted as they were considered atypical as compared to those obtained in 2009 and 2010. The results for lettuce in 2010 were also omitted, as planting of two different cultivars in alternate rows led to different development of the cultivars and atypical yields.

Marketable yield, DM of yield (DM_{yield}), and total above-ground plant material (including tubers for potato) (DM_{total}), total N uptake of above-ground plant material (including potato tubers) (N_{total}) were recorded for broccoli and potato. Soil mineral N contents (N_{soil}) in the 0–0.3 and 0.3–0.6 m soil layers were measured before planting and after harvest. Harvesting criteria and determination of yield, DM and N contents are described by Øvsthus et al. (2015, 2017).

2.6 Statistical evaluations

The goodness of fit between simulated and measured C and N mineralization values in the calibration experiment and prediction of observed crop data in the field trial were evaluated statistically. In the field trial, each crop was considered individually (not as a whole rotation). The evaluation included yield, DM, and N contents for each replicate and two years. To evaluate both the model calibration and the prediction of data from the field trial, mean absolute error (MAE) (Willmott, 1982), root mean squared error (RMSE) (Willmott, 1982), model efficiency (ME) (Nash and Sutcliffe, 1970), and coefficient of residual mass (CRM) were chosen as indices:

$$\mathrm{MAE}=\frac{\frac{1}{n}\sum_{i=1}^{n}|P_i-O_i|}{\bar{O}_n} \quad \text{(equation 4)}$$

$$\mathrm{RMSE}=\frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(P_i-O_i)^2}}{\bar{O}_n} \quad \text{(equation 5)}$$

$$\mathrm{ME}=1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{\sum_{i=1}^{n}(O_i-\bar{O}_n)^2} \quad \text{(equation 6)}$$

$$\mathrm{CRM}=\frac{\frac{1}{n}\sum_{i=1}^{n}(P_i-O_i)}{\bar{O}_n} \quad \text{(equation 7)}$$

where P_i is the simulated or predicted value and O_i is the measured or observed value at the i^{th} sampling instance (i = 1, 2, …, n), and $\bar{O}_n$ is the average of observed values. In the calibration experiment, O_i is the average of three replicates whereas in the model evaluation experiment, O_i represents each of three replicates. Additionally, for the field experiment, the percentage bias was calculated:

$$\%\ \mathrm{bias}=(O_i-P_i)*100\%/O_i \quad \text{(equation 8)}$$

MAE and RMSE include the difference between simulated and measured values, and the closer they are to zero, the better is the goodness of fit. ME compares the difference between simulated and measured values against the variance of the measured values over a period. The value ranges from minus infinite to 1, where 1 indicates a perfect fit. If the values are negative, the simulated results are worse than using the mean of the measured data. CRM and % bias indicate a tendency to overestimate (positive values) or underestimate (negative values) the measured data. For a perfect model fit the values should be equal to zero. During the calibration, achieving the values of MAE<0.3, RMSE<0.3, ME>0.5 and –0.3<CRM<0.3 were considered acceptable and further parameter adjustment was then stopped. For evaluation of the predictions of measured data in the field trial, the same values of the statistical indices were used.

3. RESULTS

3.1 Incubation of organic resources in soil at 15°C and constant temperature

During incubation of the organic resources (Table 1) in soil, initial C mineralization differed substantially between treatments but eventually converged towards slower rates after about 20 days. Overall, mineralization of added C, as calculated by the difference method, ranged from –10 to 68% after 60 days (Figure 1a). For N mineralization, the materials fell into the following main categories (Figure 1b): 1) SL, LD and AM were initially immobilizing mineral N, followed by a slow release after 10 days for SL and LD but not AM, 2) SSM, SSP, MBM and FW were initially releasing mineral N rapidly, followed by a decline in release rate after 20 days, and 3) AD and SM shows instantly high availability of mineral N with little change during the incubation. After 60 days, 40 to 80% of the added N was present as mineral N for all materials except LD (16%), SL (9%) and AM (–25%). There was a significant negative relationship (Figure 2; R^2=0.93) between the C:N ratio of the organic amendment and the N mineralization (expressed as % of added N) after 60 days.

3.2 Model calibration with measured C and N mineralization data

With some exceptions, initialization and calibration of the N mineralization module of EU-Rotate_N produced reasonably good fits with the observed C and N mineralization (Table 5 and Figure 4). For SL, LD and AM, the ME values indicated satisfactory calibrations for C mineralization (ME value ranged from 0.90 to 0.99). Figure 4 illustrate satisfactory ME values for N mineralization for SL (ME=0.53) and LD (ME=0.69), but negative ones for AM. However, the MAE and RMSE values for N mineralization were far from zero for all seaweed tested. For N-rich organic resources originating from industry (MBM, SSP, SSM and FW), MAE, RMSE and CRM were close to zero and ME close to 1 (Table 5), however, for SSP there was poor correlation (ME=0.04) between measured and simulated C mineralization (cf. Figure 4). It was difficult to calibrate the decay rate constants and C:N ratios for some of the other materials to match the measured C and N mineralization equally well. Calibration of SM resulted in a satisfactory fit with measured C mineralization (ME=0.97), but correlation indices for N mineralization were poor (ME=–5.51). For AD, the opposite was the case, with poor fit with C data (ME=–0.37). In unamended control soil, C mineralization, measured as accumulated evolution of CO_2-C, was slightly underestimated, particularly towards the end of the experiment (Figure 3). The measured mineral N in control soil was underestimated already on day zero, and the further accumulation of mineralization was so as well.

3.3 Evaluation of model performance against crop data from the field trial

Predicted and mean observed values for broccoli and potato yield, DM of yield (DM_{yield}) and total plant material (DM_{total}), N in the entire plant (N_{total}), and soil mineral N (N_{soil}) are presented in Table 6 and for broccoli fertilized with 80 kg N ha^{-1} in Appendix Table A1. The statistical indices describing goodness of fit are given in Table 7 and Appendix Table A2. The measured values for broccoli responded significantly to the type of organic resource and the N fertilizer rate, whereas potato did not. The yields were within the expected range for both crops and are presented in detail by Øvsthus et al. (2015 and 2017). The adjustment of critical %N (see the Materials and Methods section) improved the statistical agreement for potato. ME-values N_{total}, DM_{yield} and DM_{total} were improved from negative to positive (0.34, 0.44 and 0.39). For broccoli, when using default critical N% values, ME values ranged from 0.53 to 0.62 for DM_{yield}, DM_{total} and N_{total}.

In general, the model tended to underestimate the observed potato and broccoli data, as indicated by negative CRM values. Broccoli and potato fertilized with mineral fertilizer, AD, SSP and SM, and some of the AM-fertilized potato had MAE and RMSE values close to zero (lowest for mineral fertilizer, AD, SSP). Also, the correlation indices (ME) for AD, SSP and SM showed approximately the same patterns as for broccoli and potato with mineral fertilizer, and for AM in potato. For unfertilized (NF) broccoli, there was a substantial lack of fit, but the predictions of observed potato values were satisfactory.

The percentage bias (equation 8) between predicted and observed values for fresh-weight yield was 19% for broccoli fertilized with mineral fertilizer at 170 kg N ha^{-1}, while for potato at 80 kg N ha^{-1}, it was 11% (Table 7). The corresponding bias values for the organic fertilizers ranged from 1 to 49% in the order of AD<SSP<SM<AM<NF for broccoli and from 2 to 21% in the order of AD=SM<SSP<AM<NF for potato. The bias of DM_{total} ranged from 2 to 80% (lowest for AD and highest for AM) and from 0 to 26% (lowest for SM and highest for unfertilized) for broccoli and potato, respectively. Other noteworthy biases were found for potato and for N_{soil} in the case of broccoli, all of which were poorly predicted. These bias observations between predicted and observed values were also reflected in the other statistical indices.

4. DISCUSSION

4.1 Model calibration with measured C and N mineralization

The markedly different patterns of C and N mineralization from the organic materials fell into three groups similar to those identified by Jensen et al. (2005) in a similar, but more comprehensive study on plant residues. The first group consisted of the very N-rich materials of industrial origin (MBM, SSP, SSM and FW), which showed high initial C and N mineralization rates in accordance with results obtained in experiments with similar organic materials (Brod et al. 2012, 2014, 2017; Jeng et al. 2004, 2006; Thuries et al. 2001, Cayuela 2009). The calibrations were successful for MBM, SSM and FW, but it was difficult to match simulated with measured C mineralization for SSP, as the model does not explicitly include effects of physical quality of the organic materials other than indirectly through fractionation into slow and fast pools and adjustment of their decay rate constants. Despite being similar in chemical composition, the pelleted shrimp shell product SSP showed lower initial C mineralization rate than the powdered SSM. Also, N mineralization differed. These differences can most likely be explained by the physical properties of the pellets compared to those of powder. Pellets has a much smaller surface area, which most likely makes pellets more resistant to microbial attack. Moreover, pellets may create concentrated hotspots of organic material in the soil, which may lead to locally anoxic conditions favoring N dissimilation by denitrification (Cabrera et al 1994; Breland 1994; Johansen et al. 2019).

The second group of organic materials comprised the brown algae materials, which showed initial immobilization of N followed by a slow mineralization. The partitioning of C to the fast pool AOMf, guided by the amounts of structural compounds in brown algae as taken from the literature (Øverland et al. 2017; Schiener et al. 2015), seems to be adequate for SL and LD, however, not for AM. The decay rate constants for AOMf estimated by calibration ranged from 0.005 to 0.100, lowest for AM and highest for LD. The low k values for AM are atypical, whereas the estimates of the decay rate constants for SL and LD are similar to the values used for plant residues with low decomposability (Mueller et al. 1998; Neergaard et al. 2002). The atypically low value for AM may be due to biochemical properties not accounted for, but N-limitation may also be a factor, as very low concentrations of inorganic N were measured in soil with AM. Henriksen and Breland (1999c) found that C mineralization from straw was substantially reduced when soil inorganic N became depleted by microbial immobilization and introduced in their model a rate-modifying factor reducing the decay rate constant of structural material (cellulose and hemicellulose) under N-limiting conditions. The EU-Rotate_N model has a similar routine, but it might not be restrictive enough for the conditions in our experiment. The chosen pool sizes and calibrated decay rate constants resulted in satisfactory simulation of cumulative CO_2-C evolution from SL and LD, but not from AM (Figure 4). The atypically low k value that had to be set for AOMf of AM in order to match C mineralization towards the end of the incubation period, resulted in a linear increase in amount of simulated C mineralization, whereas the measured values showed curvilinearity. This is consistent with the assumption that C mineralization from AM was N-limited after depletion of soil inorganic N and that the model's factor for modifying the decay rate due to N limitation may not have been restrictive enough. Simulated N mineralization from LD and SL visually showed very good fits with measured values (Figure 4). However, the statistical indices of goodness of fit were poor. The reason is that the observed values (O_i) represent or are included in the denominator of the formulae of the statistical indices (equations 4–8), and the low values for N mineralization from LD and SL, therefore, rendered their indices more sensitive to experimental error than for treatments where observed values were higher. For AM simulated values were less negative than measured values, probably because of the low value of the AOMf decay rate constant set to match the values of accumulated C mineralization at the end of the incubation period. In addition to a likely effect of different availability of immobilizable N, as suggested above, the observed differences in C and N mineralization between AM, SL and LD were likely due to species-specific differences in chemical composition (Schiener et al. 2015), e.g., the content of polysaccharides (laminarin, mannitol, alginate, fucoidan, cellulose), monosaccharides, polyphenols, protein, ash, and total C and N. Of these, the contents of laminarin and polyphenol are higher in SL compared to LD, and alginate contents are lower in SL (Schiener et al. 2015). Studies of animal digestion of brown algae suggest that a high content of polysaccharides renders the material more recalcitrant, especially in combination with phenolic compounds (Øverland et al. 2017). This might explain the lower decay constant for SL compared to LD, despite lower C:N ratio for SL.

The third group of organic materials contained SM and AD, which in absolute terms showed instantly and persistently low C mineralization rates and high mineral N availability, especially of NH_4^+-N. Expressed as percentage of added C, however, the rate of C mineralization from AD was relatively high, which is consistent with the finding that AD application to soil often leads to microbial immobilization of mineral N (Brod et al. 2017; Alburquerque et al. 2012), although no significant immobilization was observed in the present trial. Thereafter, there was a period with less CO_2 emission in AD-treated than in the control soil, leading to "negative" C mineralization for AD. This might be due to bicarbonate build-up in the AD-treated soil, which likely had a higher pH than the control soil and possibly stimulated nitrification consuming some of the produced CO_2. Moreover, small differences in C mineralization between soil with AD and control soil after the initial CO_2 flush, rendered the estimated C mineralization from AD, which was calculated by the difference between AD-treated and control soils, vulnerable to experimental error, as partly evidenced by relatively large spread of measured values for AD (Figure 1a). Therefore, the partitioning of C between AOMs and AOMf for AD were set at the model's default values for animal manures and slurries. For SM a somewhat larger AOMf fraction was chosen because of its content of straw. The relatively good fit between simulated and estimated C mineralization suggests that this was a right decision, but for SM, the simulated mineral N values initially are lower than the measured values. This gap might be explained by different handling and storage of manures sent to analysis and manure incubated. Some N mineralization likely took place in SM between the sampling for chemical analysis, which is the basis for the mineral N in the input file, and the start of the incubation.

The underestimated N mineralization values for unfertilized control soil might be due to N mineralization during the storage period. In unamended control soil, C mineralization, measured as accumulated evolution of CO_2-C, was slightly underestimated, particularly towards the end of the experiment (Figure 3). The measured mineral N in control soil was underestimated already on day zero, and the further accumulation of mineralization was so as well.

4.2 Performance evaluation of the calibrated model

The yield and N uptake data of broccoli and potato used for the current evaluation experiment are discussed by Øvsthus et al. (2015; 2017). The EU-Rotate_N model predicted the observed values for crop growth, N uptake and yield quite well for broccoli using the original default values for critical %N for optimal crop growth. The ME values for broccoli with mineral fertilizer were comparable to those obtained in previous evaluations of the model performance (e.g., Nendel et al. 2013). However, the potato yield and the other crop data could not be predicted with the model's default values for critical %N, as the model underestimated these values for all fertilizer treatments, including the predictions obtained by using the non-calibrated values for mineral fertilizer (data not shown). The adjustment of critical %N for potato increased the model's ability to simulate the potato crop variables. This approach has been used in other model evaluations (e.g., Sun et al. 2013). In an earlier model evaluation conducted in Norway, the use of default values of critical %N resulted in simulated values of yield that corresponded well with measured values for potato (Hugh Riley, personal communication). However, the critical %N for optimum growth may vary between cultivars. 'Troll' is a potato cultivar that grows fast and gives large yields with small inputs. Therefore, it seems reasonable that it can grow with a lower N supply rate and, thus, have a lower critical %N than other potato cultivars commonly grown in Norway. In other evaluation experiments with the EU-Rotate_N model, the model predictions have also been improved by adjusting parameters related to crop growth and critical %N for optimum growth both in field and greenhouse experiments (Sun et al. 2012; Soto et al. 2018; Suarez-Rey et al. 2016; Guo et al. 2010). Our field experiment was conducted at 67.28 N and in colder climate than in other regions where the model has been tested. It is possible that crop production at this latitude and temperature may require lower critical %N for optimum growth. However, this hypothesis has not been tested scientifically.

Provided that the adjustment of the model's critical %N for potato was justified, the model predicted the yield and crop variables quite well and better than it did for the soil N variables. The deviations between predicted and observed values were acceptable for AD, SSP and mineral fertilizer. These results are within the range of other statistical evaluations of the model (Nendel et al. 2013; Rahn et al. 2010; Soto et al. 2018). Nendel et al. (2013) similarly found that the model satisfactorily predicted DM and N contents of crops, but soil mineral N predictions were poor. The underestimation of soil mineral N in the present study is in accordance with other studies (Soto et al. 2018; Doltra and Muñoz, 2016). The poor correlation for AM in the evaluation experiment was in line with the poor fit (Table 6 and 7) between simulated and measured C and N mineralization under controlled temperature and moisture conditions (Figure 4 and Table 5). For AD, the model prediction of crop data was relatively insensitive to the setting of pool fractions and estimation of C:N ratio in the input file and to the estimated values of the decay constants. This is because AD is a highly processed material with little decomposable C remaining and most of its N already present in inorganic form and, therefore, low C and N mineralization rates. For SM-fertilized potato and broccoli, the poor correlation between predicted and observed values may be caused by difficulties in finding homogenous fertilizer materials for both calibration and evaluation experiments.

The DM target yield input in the model is crucial for the accuracy of the model prediction. This DM target yield approach is based on the earlier models, such as N-ABLE and WELL_N (Greenwood 2001). In the current evaluation experiment, the measured total DM yields for broccoli and potato in the various years were used to determine the DM target yield. The need to accommodate for seasonal variation in DM target yields has been suggested earlier for improving model performance (e.g. Suárez-Rey et al 2016). This confirms the sensitivity of the model to values of input variables and illustrates that models must be used with caution, maybe in combination with other models, as a decision support tool (Palosuo et al. 2010; Rötter et al. 2012).

Model performance may also be affected by other factors than the model itself, such as pests, diseases, weeds and other factors influencing crop growth and development. However, underestimation rather than overestimation of the observed crop values makes this an unlikely cause of lack of fit in the current study. The underestimation might rather be explained by either underestimation of N mineralization or an excessively high critical %N curve. In the model, both will contribute to N-limited crop growth. In the case of AM, overestimation of N mineralization was certainly the major explanation for the poor fit between predicted and measured values.

5. CONCLUSIONS

Based on their C and N mineralization patterns, the investigated organic resources fell into three groups: organic materials of industrial origin with high N concentrations (rapid initial C and N mineralization followed by much slower one after 20 days), brown algae (moderate C mineralization and initial N immobilization followed by a slow net N release) and digestates/manure (low C mineralization and initially high mineral N content and slow or non-detectable incubation mineralization). After 60 days of incubation, 40 to 80% of added N was present as mineral N for organic materials of industrial origin, digestate and manure, whereas N mineralized from algae ranged from –25 to16% of added N. There was a significant negative relationship between increasing C:N ratio and the amount of mineral N.

For N-rich materials of industrial origin, the calibration of the EU-Rotate_N model with measured C and N mineralization at constant temperature and moisture was good. For shrimp shell pellets (SSP), which represented this group of fertilizer materials in the model evaluation experiment, the model predicted the crop data and plant N content well, but not mineral soil N data. The EU-Rotate_N model should be further improved to include physical properties in addition to chemical properties of the organic materials.

For the brown algae LD and SL, model calibration with C and N mineralization data produced good fits with measured data, but poorer ones for AM. As AM represented this group in the evaluation experiment, the crop and soil data were poorly predicted. We therefore need more knowledge about brown algae decomposition including effects of N limitation before including them in the model.

For SM, the model could be satisfactorily calibrated with measured C mineralization, but the ability to simulate N mineralization remained poor. For AD it was opposite, with poor fits for C mineralization and satisfactory fits for mineral N, which remained at a high and stable level throughout the incubation period. Model evaluation performance on crop data and N content in plants after AD fertilization was good, but the predictions of soil N data were poor.

The newly calibrated EU-Rotate_N model can be used as a tool for understanding the decomposition mechanisms which are relevant for organic materials as fertilization resource. However, as a decision tool for fertilizer management for optimum yield, economic outcome and environmental impact, it should be used in combination with other models. The model predicted yield and crop data quite well after fertilization with organic resources of industrial origin and AD, however, soil N was difficult to predict. The model needs further development before we can recommend it as decision tool for fertilization with seaweed. Still unresolved challenges that reduces the model's value as a decision support tool is the need for setting a target yield and the supposedly variable values of critical %N among different crops and possible growing conditions.

ACKNOWLEDGEMENT

We are grateful to the Norwegian Research Council (NRC), the counties of Nordland and Troms, and the Norwegian institute of Bioeconomy Research for financial support of the project – Pre- and postharvest quality optimisation of organic vegetables that can stimulate an increased consumption (NFR 176767). P. Dörsch was supported by the NRC project AGROPRO – Agronomy for increased food production in Norway – challenges and solutions (NFR 2255330/E40). Trond Knapp Haraldsen is thanked for providing MBM, FW, AD and Tor Johansen for SSM and SSP.

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Table 1. Dry matter (DM), total organic carbon (TOC), total Kjeldahl-N (TKN), ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N) and C:N ratio of the organic resources.

	pH (H_2O)	**DM (%)**	**TOC (g kg^{-1} DM)**	**TKN (g kg^{-1} DM)**	**NH_4^+-N (g kg^{-1} DM)**	**NO_3^--N (g kg^{-1} DM)**	**C:N ratio**
Shrimp shell pellets **(SSP)**	9.2	91.8	288	71.0	0.3	<0.1	4
Shrimp shell powder **(SSM)**	9.4	93.2	297	73.4	6.5	<0.1	4
Commercial algal meal **(AM)**	6.0	89.5	336	12.0	0.1	<0.1	28
Algal meal *Laminaria digitata* **(LD)**	6.4	90.3	338	18.3	0.1	0.3	19
Algal meal *Saccharina latissima* **(SL)**	6.4	90.5	342	22.2	0.3	0.8	15
Fish sludge waste **(FW)**	5.7	86.0	450	69.0	2.6	<0.1	7
Meat bone meal **(MBM)**	6.5	94.2	432	91.6	0.4	<0.1	5
Anaerobically digested food waste **(AD)**	8.6	0.85	286	676.0	619	<0.1	0.5
Sheep manure **(SM)**	8.8	15.0	336	33.7	8	<0.1	10

Table 2. Parameters values for the organic resources included in the EU-Rotate_N model calibration. Pool fractions are based on literature knowledge, and decay constants and C:N ratios are calibrated based on measured C and N mineralization from the organic resources (for explanation of their abbreviations, see Table 1).

Parameters	Units	SSP	SSM	MBM	FW	AM	SL	LD	AD	SM
Part_S (AOMs)	% of added materials	28	28	38	28	65	65	65	72	65
Part_F (AOMf)	% of added materials	72	72	62	72	35	35	35	18	25
K_Slow (AOMs)	day^{-1}	0.0002	0.0001	0.0001	0.0005	0.0001	0.0001	0.005	0.0001	0.004
K_Fast (AOMf)	day^{-1}	0.120	0.200	0.100	0.130	0.005	0.070	0.100	0.150	0.080
C:N ratio of AOMs		2.0	2.5	6.0	4.0	21.0	12.0	13.5	2.0	20.0
C:N ratio of AOMf		6.8	6.1	4.4	9.3	78.4	36.7	62.9	0.6	6.4

Table 3. Input variables used in EU-Rotate_N for model calibration and performance evaluation

Input variable	Unit	Value
Site properties		
Latitude		67.28
Altitude		35
Soil properties		
Sand (1^{st} layer)	%	91
Sand (2^{nd} layer)	%	95
Sand (3^{rd} layer)	%	95
Clay (1^{st} layer)	%	2
Clay (2^{nd} layer)	%	1
Clay (3^{rd} layer)	%	1
pH (all layers)		6.1
Bulk density (all layers)	g m^{-3}	1370
Total Carbon	g kg^{-1} DM	21
Total Nitrogen	g kg^{-1} DM	1.7
C:N ratio		12.4
Initial Mineral N	mg kg^{-1}	10.9
Organic Matter in soil (all layers)	DM	3.8
Soil moisture content		0.29
Soil moisture content		0.23
Soil moisture content		0.19
Mineral N (1^{st} layer, measured in field)	kg ha^{-1}	23
Mineral N (2^{nd} layer, measured in field)	kg ha^{-1}	9
Mineral N (3^{rd} layer, same information as 2^{nd} layer)	kg ha^{-1}	9
Physical soil properties		
Readily evaporable water (calculated after Allen et al 1998)		9
Evaporation		0.05
Drainage coefficient (unknown)		0
Vol.% water at Field Capacity (1^{st} layer)		30
Vol.% water at Field Capacity (2^{nd} layer)		17
Vol.% water at Field Capacity (3^{rd} layer)		12
Vol.% water at Permanent wilting point (1^{st} layer)		9
Vol.% water at Permanent wilting point (2^{nd} layer)		6
Vol.% water at Permanent wilting point (3^{rd} layer)		5
Vol.% water at Saturation (1^{st} layer)		48
Vol.% water at Saturation (2^{nd} layer)		50
Vol.% water at Saturation (3^{rd} layer)		49

Table 4. Day of the year (DOY) for field management operations (tillage, fertilization, planting, harvesting and sampling) at Vågønes. Data were used in the input files for the evaluation experiment.

	Year	**ploughing**	**Rototill & harrowing**	**Soil sampling spring**	**Soil sampling autumn**	**Fertilization**	**Transplanting**	**Harvesting**	**Target total plant DM* yield**
Potato	2009	158	159	145	275	160	160	274	11.6
Broccoli	2009	158	158	145	235	159	160	226	5.7
Potato	2010	158	158	132	275	160	160	274	9.0
Broccoli	2010	140	140	132	275	160	161	219	3.9

*Dry matter

Table 5. Summary of statistical parameters (see section 2.6 for explanation) for goodness of fit between simulated and measured values of C and N mineralization (kg ha^{-1}) from nine incubated organic resources and control soil (NF), as obtained by calibrating EU-Rotate_N. Values in boldface indicate that the simulation was deemed unsatisfactory according to the criteria listed in section 2.6. For explanation of the abbreviations of the organic resources, see Table 1.

Resources	Variables (unit)	MAE	RMSE	ME	CRM
Scrimp shell pellets (SSP)	CO_2-C (kg ha^{-1})	**0.50**	**0.54**	**0.04**	**0.50**
	Mineral N (kg ha^{-1})	0.12	0.14	0.93	0.10
Scrimp shell powder (SSM)	CO_2-C (kg ha^{-1})	0.12	0.16	0.86	0.10
	Mineral N (kg ha^{-1})	0.14	0.20	0.85	0.10
Meat bone meal (MBM)	CO_2-C (kg ha^{-1})	0.09	0.12	0.93	–0.03
	Mineral N (kg ha^{-1})	0.08	0.09	0.96	–0.05
Fish sludge waste (FW)	CO_2-C (kg ha^{-1})	0.13	0.14	0.91	–0.13
	Mineral N (kg ha^{-1})	0.17	0.19	0.79	–0.17
Commercial algal meal (AM)	CO_2-C (kg ha^{-1})	0.13	0.14	0.90	–0.13
	Mineral N (kg ha^{-1})	**–0.75**	**–0.82**	**–0.56**	**–0.66**
Algal meal *Saccharina latissima* (SL)	CO_2-C (kg ha^{-1})	0.07	0.08	0.98	0.03
	Mineral N (kg ha^{-1})	**4.04**	**5.37**	0.53	–0.21
Algal meal *Laminaria digitata* (LD)	CO_2-C (kg ha^{-1})	0.04	0.05	0.99	0.00
	Mineral N (kg ha^{-1})	**1.22**	**1.54**	0.69	0.07
Anaerobically digested food wastes (AD)	CO_2-C (kg ha^{-1})	**0.66**	**0.93**	**–0.37**	0.06
	Mineral N (kg ha^{-1})	0.13	0.23	**–0.53**	–0.12
Sheep manure (SM)	CO_2-C (kg ha^{-1})	0.10	0.12	0.97	0.10
	Mineral N (kg ha^{-1})	0.23	0.27	**–5.51**	–0.23
No fertilizer (NF)	CO_2-C (kg ha^{-1})	0.19	0.26	0.90	–0.20
	Mineral N (kg ha^{-1})	**0.41**	**0.41**	**–1.09**	**–0.40**

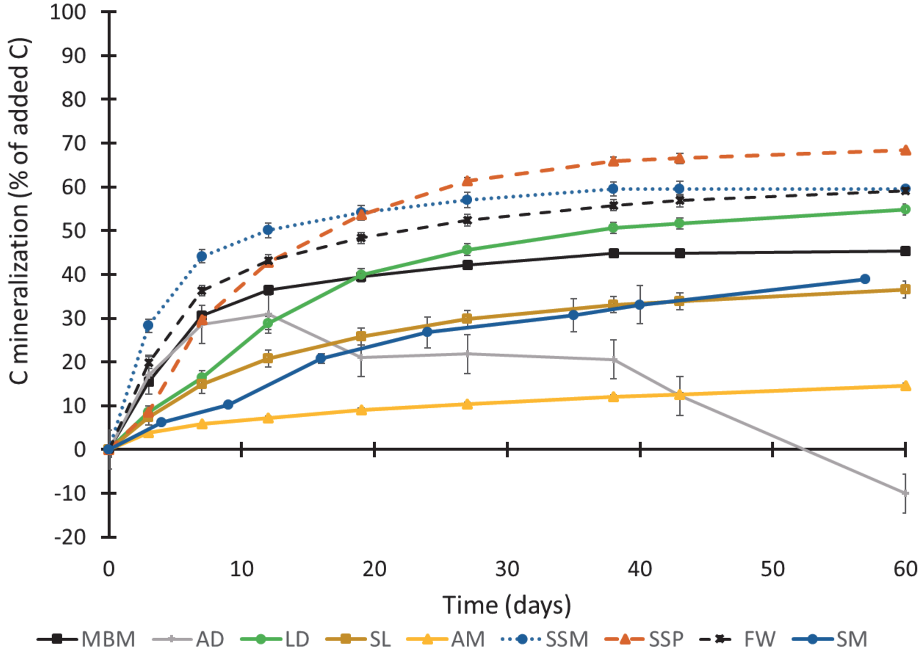
Table 6. Observed (O) and predicted (P) values for fresh-weight yield, DM yield (DM_{yield}) and DM of total above-ground plant materials including tubers for potato (DM_{total}), and N content in plant biomas (N_{total}) and mineral N in soil (N_{soil}) for potato and broccoli without fertilizer (NF) or fertilized with 80 kg N ha^{-1} and 170 kg N ha^{-1}, respectively, of mineral fertilizer (MF) or the organic resources anaerobically digested food waste (AD), shrimp shell pellets (SSP), commercial algal meal (AM), and sheep manure (SM). Observed values are average of three replicates.

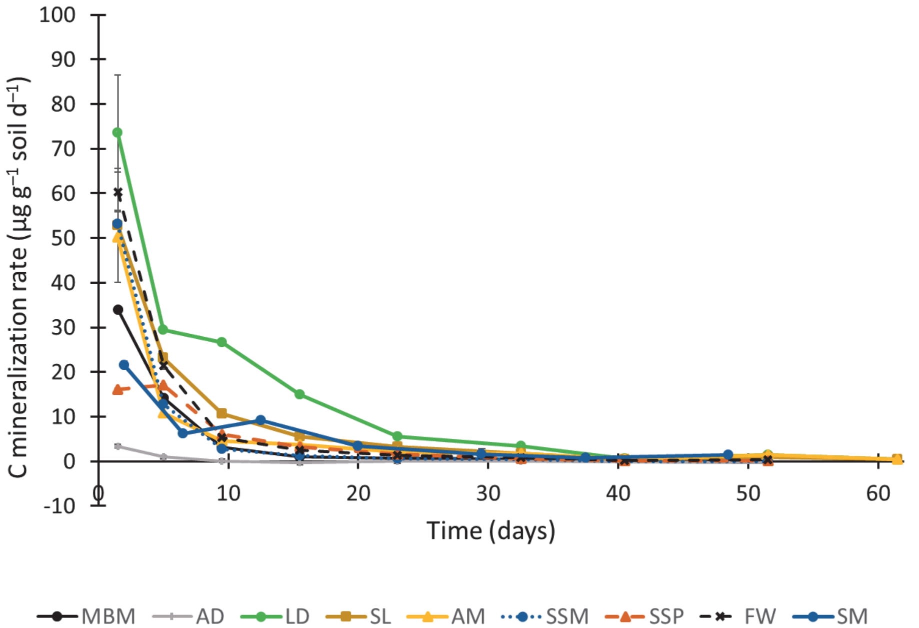
Fertilizers		**Potato 2009**		**Broccoli 2009**		**Potato 2010**		**Broccoli 2010**	
	Variables (unit)	**O**	**P**	**O**	**P**	**O**	**P**	**O**	**P**
AD	Yield (Mg ha^{-1})	41.0	40.8	10.1	10.7	31.5	30.3	6.6	6.1
	DM_{total} (Mg ha^{-1})	10.7	10.3	5.5	5.3	8.8	7.7	2.5	2.9
	DM_{yield} (Mg ha^{-1})	8.7	9.2	1.4	1.3	7.6	6.9	0.5	0.7
	N_{total} (kg N ha^{-1})	139	138	169	186	99	122	80	103
	N_{soil} (kg ha^{-1})	ND	6.0	50	12	24	43	99	79
SSP	Yield (Mg ha^{-1})	46.6	38.9	9.8	9.9	31.5	29.5	7.4	6.0
	DM_{total} (Mg ha^{-1})	12.0	9.8	5.9	4.6	8.2	7.4	3.0	2.8
	DM_{yield} (Mg ha^{-1})	9.9	8.8	1.2	1.2	7.3	6.7	0.7	0.7
	N_{total} (kg N ha^{-1})	143	124	162	144	91	120	92	95.6
	N_{soil} (kg ha^{-1})	ND	6.1	19	11	25	25	31	46
SM	Yield (Mg ha^{-1})	40.1	38.9	6.1	9.7	29.9	29.5	5.9	5.8
	DM_{total} (Mg ha^{-1})	9.9	9.8	4.8	4.5	7.3	7.4	2.6	2.6
	DM_{yield} (Mg ha^{-1})	8.3	8.8	0.8	1.2	6.6	6.7	0.5	0.7
	N_{total} (kg N ha^{-1})	111	125	107	139	73	120	72	89
	N_{soil} (kg ha^{-1})	ND	6.1	14	11	20	25	24	48
AM	Yield (Mg ha^{-1})	38.9	26.1	3.2	1.9	15.2	19.0	1.2	0.9
	DM_{total} (Mg ha^{-1})	8.2	6.4	4.8	0.8	3.2	4.7	1.1	0.4
	DM_{yield} (Mg ha^{-1})	6.9	5.9	0.5	0.2	2.8	4.3	0.1	0.1
	N_{total} (kg N ha^{-1})	91	71	77	24	41	69	19	10.9
	N_{soil} (kg ha^{-1})	ND	6	22	14	25	14	14	17
MF	Yield (Mg ha^{-1})	47.4	42.0	10.5	10.7	35.9	32.1	9.9	5.9
	DM_{total} (Mg ha^{-1})	11.6	10.6	5.6	5.4	9.0	8.3	3.6	2.9
	DM_{yield} (Mg ha^{-1})	9.5	9.5	1.3	1.3	8.0	7.3	0.8	0.7
	N_{total} (kg N ha^{-1})	156	145	181	199	100	126	117	104
	N_{soil} (kg ha^{-1})	ND	12	47	15	37	87	40	94
NF	Yield (Mg ha^{-1})	37.1	25.3	5.0	2.9	18.7	19.0	4.0	1.7
	DM_{total} (Mg ha^{-1})	9.7	6.2	4.1	1.2	5.0	4.7	1.8	0.7
	DM_{yield} (Mg ha^{-1})	8.1	5.7	0.6	0.4	4.4	4.3	0.4	0.2
	N_{total} (kg N ha^{-1})	107	67	88	32	53	66	48	21
	N_{soil} (kg ha^{-1})	ND	6	19	12	19	14	26	18

Table 7. Summary of statistical parameters (see explanation in section 2.6) for goodness of fit between model-predicted and observed fresh-weight yield, DM yield (DM_{yield}) and DM of total above-ground plant biomass including tubers for potato (DM_{total}), N contents in total plant biomass (N_{total}) and mineral N in soil (N_{soil}) for broccoli and potato without fertilizer (NF) or fertilized with mineral fertilizer (MF), anaerobically digested food waste (AD), scrimp shell pellets (SSP), sheep manure (SM) or algal meal (AM) at rates of 80 and 170 kg N ha^{-1} for potato and broccoli, respectively, for three replicates in 2009 and 2010 (n=6). Boldface numbers indicate poor model fit according to the criteria listed in section 2.6.

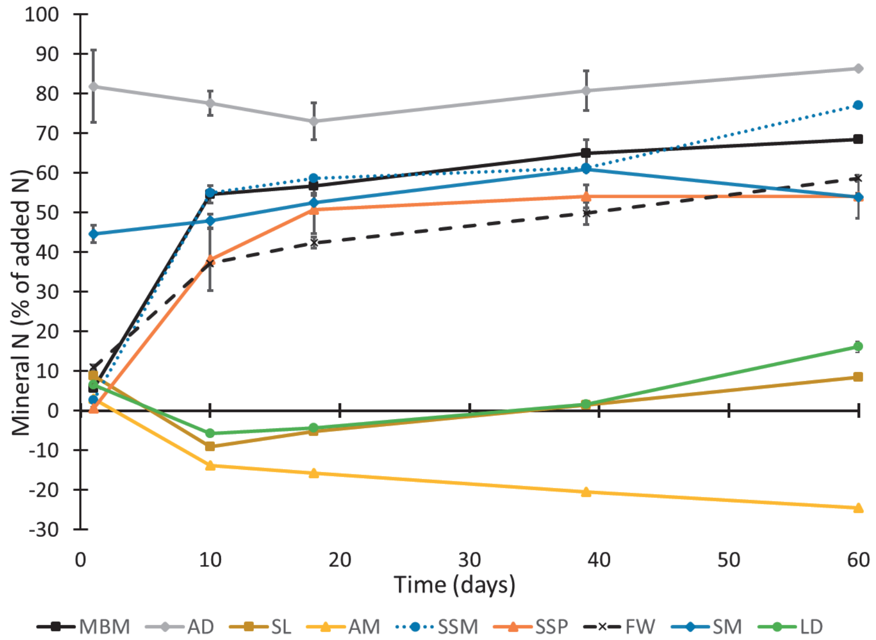
		Broccoli					Potato				
	Unit	MAE	RMSE	ME	CRM	% bias	MAE	RMSE	ME	CRM	% bias
AD	Yield (Mg ha^{-1})	0.11	0.15	0.63	0.00	–1	0.09	0.09	0.65	–0.02	2
	DM_{total} (Mg ha^{-1})	0.10	0.11	0.92	0.03	–2	0.11	0.13	**0.15**	–0.07	8
	DM_{yield} (Mg ha^{-1})	0.25	0.27	0.71	0.04	–5	0.11	0.13	**–0.03**	–0.01	1
	N_{total} (kg N ha^{-1})	0.16	0.18	0.75	0.15	–16	0.13	0.15	**0.31**	0.09	–9
	N_{soil} (kg ha^{-1})	**0.50**	**0.65**	**–0.14**	**–0.39**	39					
SSP	Yield (Mg ha^{-1})	0.26	**0.33**	**0.04**	–0.07	8	0.15	0.16	**0.38**	–0.12	12
	DM_{total} (Mg ha^{-1})	0.17	0.22	0.58	–0.16	17	0.16	0.18	**0.20**	–0.15	15
	DM_{yield} (Mg ha^{-1})	0.30	**0.40**	**0.32**	0.00	0	0.10	0.12	**0.46**	–0.1	10
	N_{total} (kg N ha^{-1})	0.14	0.20	0.63	–0.06	–11	0.20	0.25	**0.08**	0.04	–4
	N_{soil} (kg ha^{-1})	**0.46**	**0.50**	**–2.10**	0.13	–13					
SM	Yield (Mg ha^{-1})	**0.33**	**0.44**	**–15.8**	0.29	–29	0.07	0.09	0.77	0.00	2
	DM_{total} (Mg ha^{-1})	0.19	0.21	0.65	–0.05	4	0.07	0.08	0.77	0.00	0
	DM_{yield} (Mg ha^{-1})	**0.39**	**0.43**	**–2.99**	**0.39**	–46	0.08	0.1	0.54	0.04	–5
	N_{total} (kg N ha^{-1})	0.28	**0.32**	**–0.86**	0.28	–27	**0.33**	**0.39**	**–1.85**	**0.33**	–33
	N_{soil} (kg ha^{-1})	**0.70**	**0.92**	**–6.51**	**0.55**	-54					
AM	Yield (Mg ha^{-1})	**0.37**	**0.58**	**0.08**	**–0.36**	36	**0.31**	**0.36**	**0.35**	–0.17	17
	DM_{total} (Mg ha^{-1})	**0.80**	**0.99**	**–1.31**	**–0.80**	80	0.29	**0.31**	0.53	–0.02	3
	DM_{yield} (Mg ha^{-1})	**0.50**	**0.68**	**–0.26**	**–0.50**	50	0.26	**0.28**	0.58	0.06	5
	N_{total} (kg N ha^{-1})	**0.64**	**0.82**	**–0.61**	**–0.63**	64	**0.41**	**0.44**	**–0.24**	–0.41	–6
	N_{soil} (kg ha^{-1})	**0.31**	**0.51**	**–0.31**	–0.15	15					
MF	Yield (Mg ha^{-1})	0.29	**0.31**	**–3.56**	–0.19	19	0.11	0.12	**0.30**	–0.11	11
	DM_{total} (Mg ha^{-1})	0.14	0.16	0.57	–0.10	10	0.09	0.10	**0.44**	–0.09	8
	DM_{yield} (Mg ha^{-1})	0.18	0.24	0.53	–0.06	5	0.07	0.08	**0.39**	–0.04	4
	N_{total} (kg N ha^{-1})	0.11	0.15	0.62	0.02	–2	0.21	0.21	**0.34**	0.06	–6
	N_{soil} (kg ha^{-1})	**1.00**	**1.08**	**–9.23**	0.25	–25					
NF	Yield (Mg ha^{-1})	**0.49**	**0.51**	**–7.26**	**–0.49**	49	0.24	**0.32**	**0.16**	–0.21	21
	DM_{total} (Mg ha^{-1})	**0.68**	**0.75**	**–2.52**	**–0.68**	68	0.27	**0.34**	**–0.04**	–0.25	26
	DM_{yield} (Mg ha^{-1})	**0.43**	**0.46**	**–2.06**	**–0.43**	40	0.23	0.29	**0.13**	–0.19	18
	N_{total} (kg N ha^{-1})	**0.61**	**0.65**	**–3.44**	**–0.61**	61	**0.33**	**0.39**	**–0.16**	–0.16	17
	N_{soil} (kg ha^{-1})	**0.33**	**0.37**	**–1.63**	**–0.33**	33					

A)

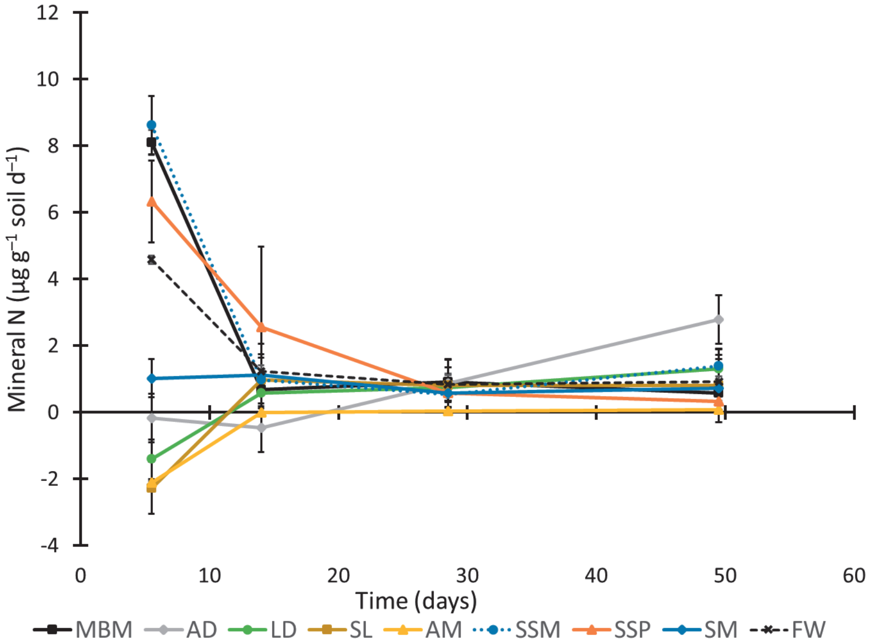

C mineralization (% of added C)
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0
10
20
30
40
50
60
Time (days)
MBM
AD
LD
SL
AM
SSM
SSP
FW
SM


C mineralization rate (µg g⁻¹ soil d⁻¹)
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
Time (days)
MBM
AD
LD
SL
AM
SSM
SSP
FW
SM

B)



Mineral N (% of added N)
Time (days)
MBM AD SL AM SSM SSP FW SM LD



Mineral N (µg g⁻¹ soil d⁻¹)
Time (days)
MBM AD LD SL AM SSM SSP SM FW

Figure 1. Carbon mineralization (% of added C) and C mineralization rate (µg g^{-1} soil d^{-1}) (A) and N mineralization (% of added N) and N mineralization rate (µg g^{-1} soil d^{-1}) (B) from the organic resources during 60 days of incubation at 15°C and constant soil moisture. Values were averaged of three replicates (n = 3) and bars indicate standard deviation. Abbreviations: SSP, Shrimp shell pellets; SSM, Shrimp shell powder; AM, Commercial algal meal; LD, Algal meal *Laminaria digitata;* SL, Algal meal *Saccharina latissimi;* FW, Fish sludge waste; MBM, meat bone meal; AD, anaerobically digested food waste; SM, Sheep manure.

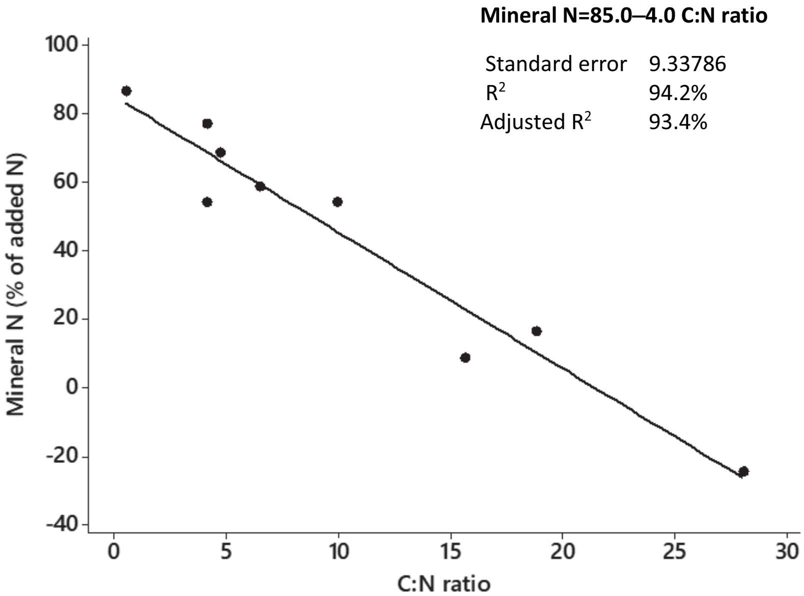


Figure 2. Correlation between C:N ratio in the organic materials and the N mineralization after 60 days.

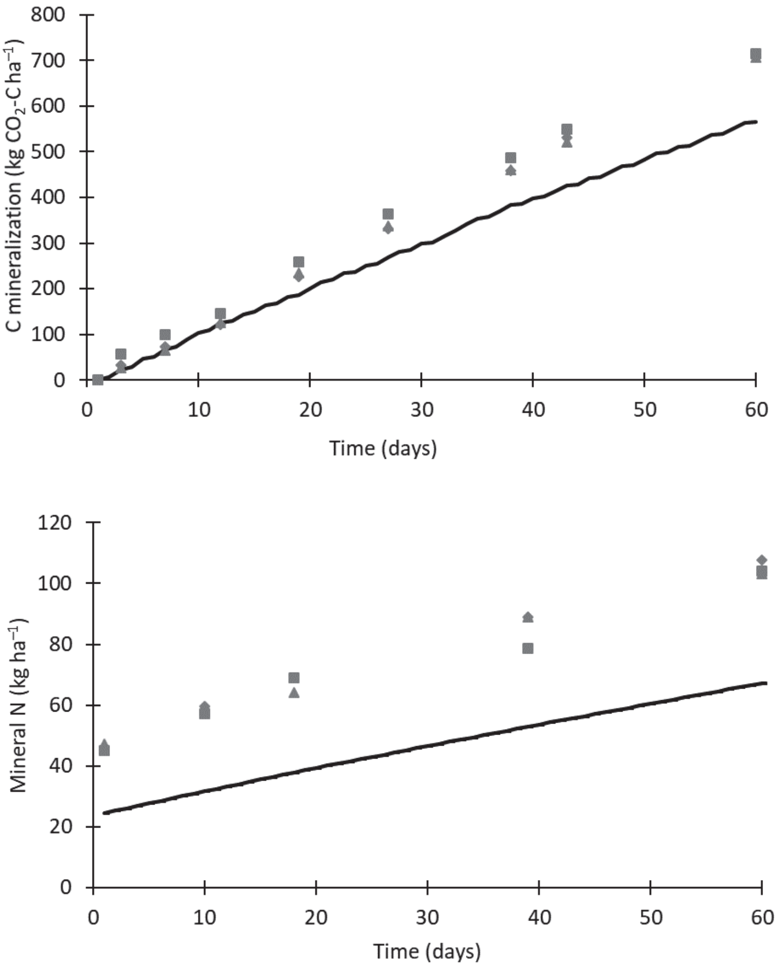
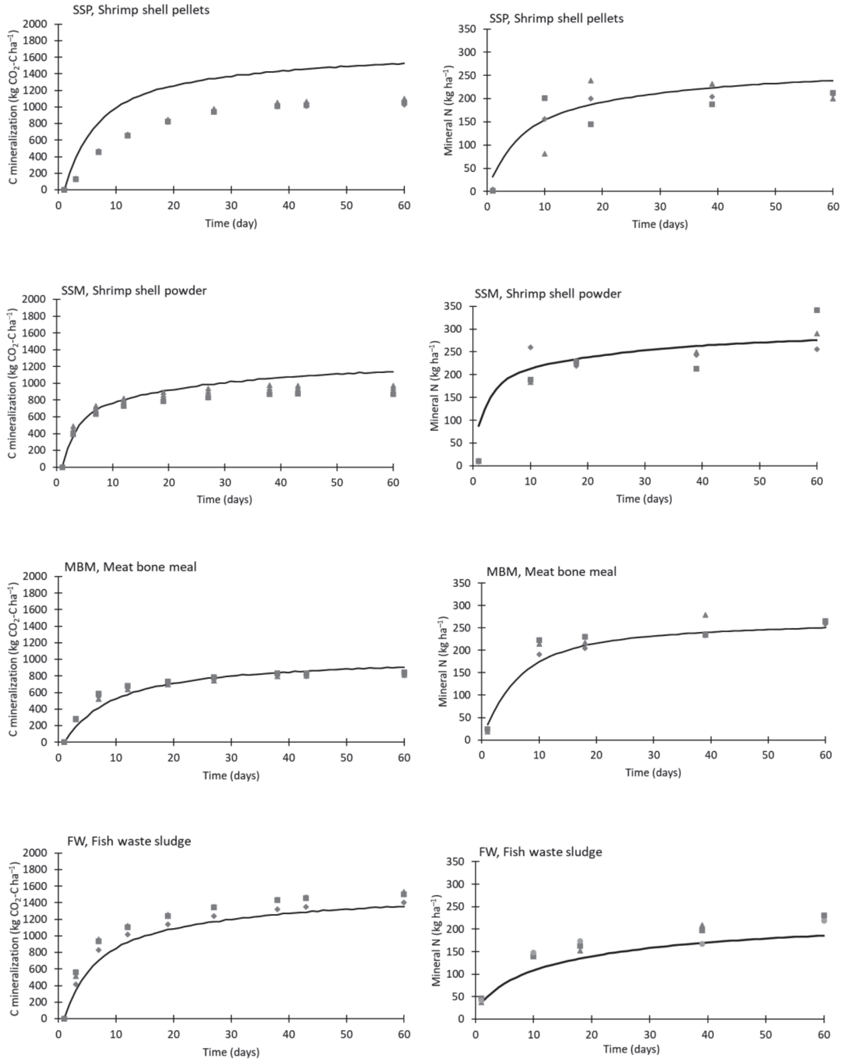


Figure 3. Simulated (lines) and measured (dots) rates of CO_2-C evolution and mineral N accumulation in soil without added organic resources.


SSP, Shrimp shell pellets
C mineralization (kg CO_2-C ha^{-1})
Time (day)
SSP, Shrimp shell pellets
Mineral N (kg ha^{-1})
Time (days)
SSM, Shrimp shell powder
C mineralization (kg CO_2-C ha^{-1})
Time (days)
SSM, Shrimp shell powder
Mineral N (kg ha^{-1})
Time (days)
MBM, Meat bone meal
C mineralization (kg CO_2-C ha^{-1})
Time (days)
MBM, Meat bone meal
Mineral N (kg ha^{-1})
Time (days)
FW, Fish waste sludge
C mineralization (kg CO_2-C ha^{-1})
Time (days)
FW, Fish waste sludge
Mineral N (kg ha^{-1})
Time (days)

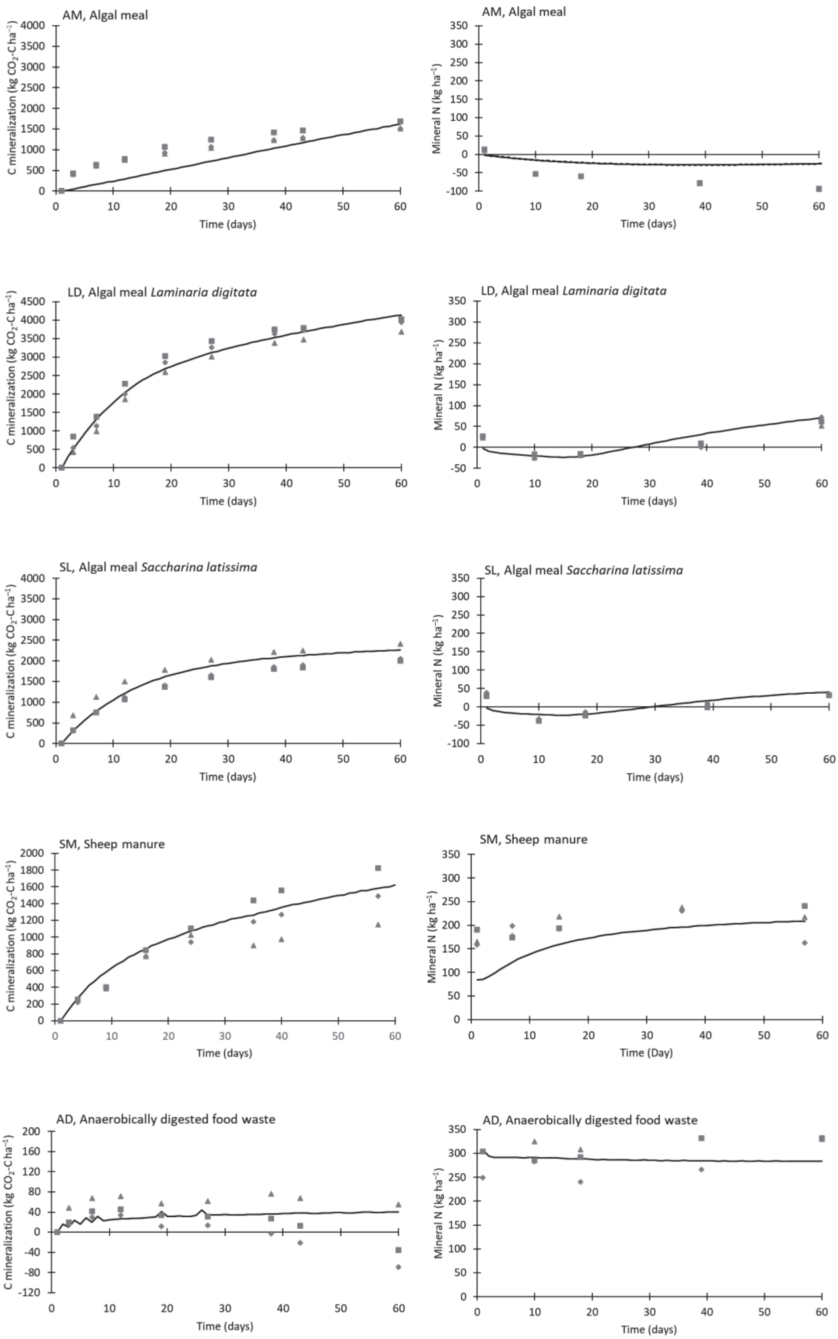


Figure 4. Measured (replication dots: □, Δ and ◊) and simulated (lines) C and N mineralization (kg ha^{-1}) from organic resources during 60 days of incubations at 15 °C and constant soil moisture.

Appendix

Table A1. Observed (O) and predicted (P) values for fresh-weight yield, DM yield (DM_{yield}), DM of total above-ground plant materials (DM_{total}), and N content in plant (N_{total}) and mineral N in 0–90 cm soil (N_{soil}) for broccoli fertilized with 80 kg N ha^{-1} of shrimp shell pellets (SSP), algal meal (AM), anaerobically digested food waste (AD) and sheep manure (SM). Observed values are average of three replicates.

Fertilizers		Broccoli 2009		Broccoli 2010	
	Variables (unit)	O	P	O	P
AD	Yield (Mg ha^{-1})	8.4	8.7	7.4	4.4
	DM_{total} (Mg ha^{-1})	5.5	3.9	2.5	2.0
	DM_{yield} (Mg ha^{-1})	1.1	1.0	0.6	0.5
	N_{total} (kg N ha^{-1})	136	108	78	61
	N_{soil} (kg ha^{-1})	16	11	31	46
SSP	Yield (Mg ha^{-1})	7.9	7.6	5.3	4.3
	DM_{total} (Mg ha^{-1})	5.5	3.3	2.4	1.8
	DM_{yield} (Mg ha^{-1})	1.1	0.9	0.5	0.5
	N_{total} (kg N ha^{-1})	135	88	73	57
	N_{soil} (kg ha^{-1})	13	11	34	31
SM	Yield (Mg ha^{-1})	5.5	7.6	4.1	4.1
	DM_{total} (Mg ha^{-1})	4.3	3.3	2.3	1.7
	DM_{yield} (Mg ha^{-1})	0.8	0.9	0.4	0.5
	N_{total} (kg N ha^{-1})	96	88	58	54
	N_{soil} (kg ha^{-1})	18	11	23	31
AM	Yield (Mg ha^{-1})	4.3	2.8	1.7	1.3
	DM_{total} (Mg ha^{-1})	4.8	1.1	1.2	0.5
	DM_{yield} (Mg ha^{-1})	0.6	0.3	0.2	0.2
	N_{total} (kg N ha^{-1})	104	32	25	16
	N_{soil} (kg ha^{-1})	28	12	18	18

Table A2. Summary of statistical parameters (see explanation in the text) for goodness of fit between model-predicted and observed fresh-weight yield, DM yield (DM_{yield}) and DM of total above-ground plant biomass (DM_{total}), N contents in total plant biomass (N_{total}) and mineral N in soil (N_{soil}) for broccoli fertilized with 80 kg N ha^{-1} of anaerobically digested food waste (AD), scrimp shell pellets (SSP), sheep manure (SM) or algal meal (AM) for three replicates in 2009 and 2010 (n=6). Bold numbers indicate poor model fit.

		Broccoli				
	Unit	MAE	RMSE	ME	CRM	%bias
AD	Yield (Mg ha^{-1})	**0.37**	**0.43**	**–0.60**	–0.17	17
	DM_{total} (Mg ha^{-1})	0.26	**0.32**	0.35	–0.26	26
	DM_{yield} (Mg ha^{-1})	0.28	**0.36**	0.31	–0.11	12
	N_{total} (kg N ha^{-1})	0.28	**0.32**	0.20	–0.21	21
	N_{soil} (kg ha^{-1})	**0.43**	**0.50**	**–1.29**	0.23	–39
SSP	Yield (Mg ha^{-1})	0.15	0.18	0.51	–0.03	10
	DM_{total} (Mg ha^{-1})	**0.30**	**0.37**	**0.12**	–0.03	35
	DM_{yield} (Mg ha^{-1})	0.23	0.25	0.66	0.04	13
	N_{total} (kg N ha^{-1})	0.24	**0.33**	**0.16**	–0.22	30
	N_{soil} (kg ha^{-1})	0.16	0.21	0.81	–0.13	12
SM	Yield (Mg ha^{-1})	**0.40**	**0.44**	**–1.40**	0.26	–22
	DM_{total} (Mg ha^{-1})	0.23	0.29	0.33	–0.22	24
	DM_{yield} (Mg ha^{-1})	**0.33**	**0.38**	**0.00**	0.29	–17
	N_{total} (kg N ha^{-1})	0.10	0.11	0.85	–0.01	8
	N_{soil} (kg ha^{-1})	**0.36**	**0.43**	**–1.86**	0.06	–3
AM	Yield (Mg ha^{-1})	**0.40**	**0.53**	**–0.05**	–0.40	32
	DM_{total} (Mg ha^{-1})	**0.75**	**0.91**	**–1.31**	0.75	73
	DM_{yield} (Mg ha^{-1})	**0.48**	**0.65**	**–0.09**	–0.48	38
	N_{total} (kg N ha^{-1})	**0.66**	**0.83**	**–0.78**	–0.66	63
	N_{soil} (kg ha^{-1})	**0.34**	**0.63**	**–0.76**	–0.34	36

Paper IV

Johansen TJ, Samuelsen TA and Øvsthus I, 2019. Growth and nitrogen recovery efficiency of potato (*Solanum tuberosum*) fertilised with shrimp shell pellets. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 69(7):559–566

Acta Agriculturae Scandinavica, Section B — Soil & Plant Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

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To cite this article: Tor J. Johansen , Tor A. Samuelsen & Ingunn Øvsthus (2019) Growth and nitrogen recovery efficiency of potato (*Solanum tuberosum*) fertilised with shrimp shell pellets, Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 69:7, 559-566, DOI: 10.1080/09064710.2019.1617344

To link to this article: https://doi.org/10.1080/09064710.2019.1617344

Published online: 16 May 2019.

ACTA AGRICULTURAE SCANDINAVICA, SECTION B — SOIL & PLANT SCIENCE
2019, VOL. 69, NO. 7, 559–566
https://doi.org/10.1080/09064710.2019.1617344

Growth and nitrogen recovery efficiency of potato (*Solanum tuberosum*) fertilised with shrimp shell pellets

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ABSTRACT

In organic plant production, nitrogen (N) availability is often a growth-limiting factor. Under such conditions, off-farm waste-derived nutrient resources may be an alternative to meet the N demand. In this study, we described a production method for a shrimp shell (SS) pellet product and evaluated the N fertiliser effect and N recovery efficiency (NRE) in a controlled climate pot experiment with potatoes. The experiment was set up with low, medium and high N levels of SS pellets in comparison with a standard mineral fertiliser (MF) at 9°C, 15°C and 21°C. In a separate study, we examined the loss of N as N_2O from SS pellets in comparison with SS powder in a 100 days incubation experiment. The results documented the possibility to formulate a fertiliser pellet product from SS, and that SS pellets were an effective N fertiliser in potato at all growth temperatures. Nevertheless, a slightly slower development and lower tuber yields than for MF indicated a delayed N-availability from SS pellet fertiliser. NRE after use of MF was around 90%, and about 70% for the different levels of SS pellets. The incubation experiment showed a higher rate of available N for SS powder than for pellets (67% and 39%, respectively) after 100 days of incubation at constant humidity and temperature. This difference was attributed to a lower degree of dissolved materials and a higher rate of denitrification and N_2O emissions for pellets than for powder, probably caused by differences in physical properties, occurrence of anoxic hotspots and higher microbial activity around and inside the SS pellets.

ARTICLE HISTORY
Received 8 February 2019
Accepted 6 May 2019

KEYWORDS
Controlled climate; extrusion; nitrogen mineralisation; nitrous gas emission; pelletising; waste-derived fertilisers

Introduction

In organic plant production, nitrogen (N) availability is a growth-limiting factor, especially on stockless farms without animal manure and in cold climates with reduced decomposition of green manure and limited N-fixation by legumes. Under such growing conditions, there is often a need for off-farm nutrient resources to meet the N demand. In northern areas, there has been a special attention to utilising marine waste-derived organic materials as fertilisers (Ytreberg 1959; Bjøru 1996). Such slaughter residues are generally rich in nutrients and energy, and constituted about 914,000 Mg in Norway in 2016 (Richardsen et al. 2017). Shellfish (mainly shrimp shell) constituted about 12,000 Mg, of which about 29% was utilised as fish fodder meal, chitin/chitosan production, cosmetics, etc.

In 2003–2005, the growers association 'Ottar' in North-ern-Norway, initiated several studies on the fertiliser effect of both fresh shrimp shell (SS) and dried SS powder in greenhouse and field experiments with potatoes (Tor J. Johansen, unpublished). Chemical analyses showed that these products had a wide and relatively balanced nutrient content related to potato requirements, except for a minimal content of potassium (K). However, with supplements of K, the growth responses were comparable to the use of mineral fertiliser (MF), though with a slightly delayed N availability for fresh shells in the field experiments.

Use of fresh SS and SS powder have limited relevance for commercial use due to challenges within transport, storage and application. This management problem can be solved by processing the SS powder into pellets by use of pelletising or extrusion technology; production methods extensively used in feed and food manufacturing. In a pelletising process, a moistened and heated material is compacted and shaped through die holes into pellets and dried (Thomas et al. 1997). Extrusion is a process that transforms the material into a high viscous flowable mass controlled by water and steam injection and viscous heat dissipation in one or two

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screws. The material is then shaped through dies, cut into pellets and dried (Riaz 2000). Extruded pellets generally have higher physical quality and produce less fine particles than at pelletising. However, the durability of the final product from both processes is dependent on technical properties of the protein components and normally improved by the addition of starch and other binders (Thomas et al. 1998; Samuelsen et al. 2013; Samuelsen and Oterhals 2016).

In 2007, the grower's association 'Ottar' initiated a new project (2007–2010), including a test-production of SS pellet products and further studies of workability and fertiliser effects in field and controlled climate chambers. In an adjoining research project the chosen pellet product was tested for its effects on yield, N-contents and quality, in a field study with broccoli, potato and lettuce (Øvsthus et al. 2015, 2017). Results indicated adequate N mineralisation and effects as N fertiliser. In addition, Øvsthus et al. (2017) investigated the N-recovery efficiency (NRE, also called N-use efficiency, NUE) for the SS pellets. For potato, yields at estimated available N-levels of 80 kg ha^{-1} for the SS pellets, did not differ significantly from the similar N-level of MF, and the NRE was close to 50% for SS pellets in field, compared to around 60% for MF. The authors also showed that residuals of inorganic N in soil were at moderate or un-detectable levels, and did not differ between fertilisers at the end of season.

Before using these waste resources commercially, knowledge about their fertiliser effect (N availability) is required to predict yield and impact on the environment. The N fertiliser value of organic materials are dependent on highly unpredictable environmental factors, such as humidity, temperature and oxygen, and on the chemical quality of organic materials (e.g. C:N ratio) (Nicolardot et al. 2001; Jensen et al. 2005). In addition, synchronisation of N mineralisation with crops N demand will reduce the risk for N being lost from the soil as nitrate (NO_3^-) or as N gasses (N_2O, NO, NO_2 or N_2) from denitrification processes (Borgen et al. 2012; Hayakawa et al. 2009; Øvsthus et al. 2015, 2017). Recently, a study of pelleted compound recycling fertilisers aimed at a balanced nutrient ratio, by combining N- and phosphorus (P)-rich wastes with K-rich material (Brod et al. 2018). Results showed a good durability of the pellet product, but in this case a too low N-concentration relative to P and K according to the crop demands.

To our knowledge, there is no documentation in the published literature on the production of SS pellet fertilisers and its technical quality regarding practical use. Further, only one study in field conditions (Øvsthus et al. 2017) have focused on plant growth and NRE for SS used as fertiliser, and no studies have dealt with both N mineralisation and potential denitrification (N_2O-emissions) for this product. Therefore, this study address a method for SS pellet production, demonstrate its N-effect on plant growth in various climates, and investigate potential N losses to the environment. We do this by means of the following objectives: (1) to document the possibility to produce a SS pellet fertiliser, including technical quality descriptions, (2) to record potato growth, N-uptake and NRE at three fixed temperatures in a pot experiment with SS pellet fertiliser, and (3) to assess N_2O emissions and N mineralisation in an incubation experiment with both pellets and powder of SS.

Materials and methods

Shrimp shell pellet production

Fertiliser materials, pellet production and -quality: The SS powder was based on dried SS and heads (*Pandalus borealis*) from Bioprawns AS, Nord-Lenangen, Norway. Experimental pellet samples were produced at Nofima, Bergen, Norway. A mix containing 940 g kg^{-1} of the SS powder, 50 g kg^{-1} whole-wheat flour (Norgesmøllene AS, Vaksdal, Norway) and 10 g kg^{-1} soy bean oil (purchased locally) was prepared and homogenised. The dry mix, calibrated to 150 kg h^{-1}, were processed in an atmospheric double differential preconditioner (Wenger Manufacturing Inc., Sabetha, KS, USA) followed by extrusion on a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger). Nine circular 3.5 mm dies restricted the extruder outlet. The feed mixture was extruded with a total steam and water flow at 16.1 and 24.9 kg h^{-1}, respectively. The wet extrudates were cut at the extruder die surface to an approximate length of 4 mm and dried at 70°C in a hot air dual layer carousel dryer (Model 200.2, Paul Klöckner GMBH, Nistertal, Germany). A total of 227 kg pellet were produced, and 220 kg retained after sieving on a 2 mm screen (i.e. a process yield of 97%). The final sieved pellets were stored in closed containers at ambient temperature prior to analysis and shipment.

Initially, trial productions were performed on a pellet mill with 5 mm ring die holes (Simon Heesen, The Netherlands). However, neither process yield, nor physical pellet quality was considered as satisfying using the pellet mill in this experiment.

The following physical quality parameters of SS powder and pellets were studied: Pellet diameter was measured with an electronic calliper and based on averages of 20 pellets. Mechanical durability was measured by use of a tumbling box (Matador, Esbjerg, Denmark). A 500 g pellet sample was rotated 500 times in a rectangular box. After the test cycle, the amount of pellets remaining on a 2 mm screen was measured, and durability expressed as the weight-percentage of pellets retained. Durability are based on averages of duplicate analyses. Bulk density was measured by loosely pouring the SS powder or pellets through a funnel into a 1000 ml measuring cylinder. A dust fraction was defined for the SS powder as the percent passing through the 325 mesh sieve (<44 µm; air jet sieve Alpine A200LS-N, Hosokawa Micron Ltd., Cheshire, UK).

Pot experiment with potatoes

Experimental conditions and potato material: Experiments were carried from 25th of April to 27th of August 2008 at the phytotron of The Arctic University of Norway (UiT), located at Holt, Tromsø (69.7°N, 18.9°E). Conditions in the climate chambers were fixed temperatures (±0.5°C), natural daylight, and air humidity standardised at a water vapour pressure deficit of 0.5 kPa. The potato material was pre-basic seed tubers (about 30 g) of the medium early Norwegian cultivar Troll. Growing substrate was a 60:10:30 (v/v) mixture of (1) moist nutrient-deficient peat ('Naturtorv', natural sphagnum peat, Tjerbo Torvfabrikk AS, Rakkestad, Norway), with addition of 6 kg lime ($CaMg(CO_3)_2$, Franzefoss Bruk AS, Ballangen, Norway) per 1000 L usable volume, (2) sand (approx. 0.1–2 mm) and (3) perlite (Agra perlite, Rhenen, Netherlands, 0–6.5 mm). The pH in the substrate after liming was expected to be 5.5–6.5, similar to standard fertilised peat from the producer. Pots, with drainage openings 5 cm above the bottom, were filled with 10 L (7 kg) each of this substrate.

The extruded pellet product had a dry matter (DM) content of 90.2%, total organic carbon (TOC) content of 28.8%, C:N-ratio of 4, pH of 9.2, and a nutrient content of 7.2% N (Kjeldahl), 2.7% P, 0.1% K and 0.4% S of DM (Øvsthus et al. 2015). The ammonium and nitrate contents in the pellets were 0.3 and <0.1 g kg^{-1} DM, respectively. Due to limited content of potassium (K) and some micronutrients (eg. Mn) in SS, additional potassium sulfate (K_2SO_4, 41% K, Yara, K + S Group, Germany) and fritted trace elements (F.T.E. no. 36; Mn, B, Fe, Zn, Cu, Mo) were added separately into the growth medium. Mineral fertiliser (MF) was applied as NPK 11-5-18 (Yaramila Fullgjødsel®, Yara International, Norway).

Experimental design and treatments: The experiment was set up with five treatments; three levels of SS pellets, one level of MF and control (no fertiliser; NF) (Table 1). Total amounts of N supplied per 10 L pot (one plant) were 0.68, 1.35 and 2.03 g for treatment SS1, SS2 and SS3, respectively, and 1 g N for the MF treatment, equivalent to 100 kg available N ha^{-1} (Table 1). The N-levels for the SS treatments were aimed at an approximate equivalent to 50, 100 and 150 kg available N ha^{-1} in field application, and the N-availability for SS2 was assumed equal to MF. The calculations were based on previous experiences with potatoes in pot experiments, with an assumption of 80% N-availability for SS (Tor J. Johansen, unpublished results). Rates of the additional K and micronutrients were set at amounts corresponding to the K and Mn content in MF for SS2, ±50% for the lower and higher SS levels, respectively.

Table 1. Applied fertilisers and supplemental nutrients, and total NPK contents per 10 L pot (one potato plant). Treatments were mineral fertiliser (MF), pellets of shrimp shell powder in increasing fertiliser rates (SS1-3) and no fertiliser (control, NF). Potassium (K) was applied as K_2SO_4 and micronutrients as F.T.E. no. 36.

Applied fertiliser and nutrients per pot				Total NPK contents		
Fertiliser	Fertiliser (g)	K_2SO_4 (g)	FTE 36 (g)	N (g)	P (g)	K (g)
MF	9.1	0.00	0.00	1.00	0.42	1.62
SS1	10.4	1.95	0.68	0.68	0.31	0.81
SS2	20.8	3.90	1.36	1.35	0.62	1.62
SS3	31.2	5.85	2.04	2.03	0.93	2.43
NF	0.0	0.00	0.00	0.00	0.00	0.00

Fertilisers were mixed into the growing substrate in the upper 1/3 level of each pot and seed tubers were planted at 5 cm depth. Experiments were performed at three growth temperatures (9°C, 15°C and 21°C) with six pots for each of the five treatments at each temperature. Pots were placed on trolleys (two pots on each), and were randomly positioned within the chambers at weekly intervals. Water was supplied daily at demand (estimated), and once a week up to a defined pot weight for each treatment (7 kg + weight of the increasing plant biomass).

Growth data and chemical analyses: After planting, the time for emergence of sprouts was recorded for individual plants. Further observations were done at harvest (68, 82 and 124 days after planting, for plants grown at 21°C, 15°C and 9°C, respectively). The timing aimed at approximately similar developmental stages of the MF treatments at these growth temperatures. At harvest, the following data were recorded: percent fresh (green) haulm by subjective visual estimation, number of aboveground stems, total number of tubers (included stolon tip swellings above 10 mm), fresh matter (FM) and dry matter (DM) of total biomass (separated in haulm (aboveground stems and leaves), underground stems and roots, and tubers). Finally, FM biomass and percent DM content (based on specific gravity) of tubers were recorded. For the chemical analyses of total N content (TN) in plants (tubers, haulm, roots, underground stems and roots) after harvest, samples were combined for two and two pots (three samples per treatment). Eurofins Food and Agro Testing Norway AS performed the analyses.

N-recovery efficiency: N-recovery efficiency (NRE) is an expression of the rate N applied taken up by the plant, after subtraction for uptake from unfertilised plants (NF). Calculations were performed according to the following formula (Craswell and Godwin 1984): NRE = $(U - U_0)/N_A$, where U and U_0 are uptake of total N per plant grown with and without fertiliser, respectively. N_A is the amount of applied N per plant.

Incubation experiment

SS pellets and powder, respectively, at amounts equal to 110 mg N (corresponding to 300 kg N ha^{-1}) were incorporated in 100 g DM soil in 0.2 L open glass jars. The soil was a sandy, orthic humo-ferric podzol with pH 6.1, sampled at Vågønes, Bodø (a previous NIBIO research station). It contained 91% sand, and contents of total carbon and total N in the soil were 21 and 1.7 g kg^{-1}, respectively. The samples were incubated at 15°C at constant humidity (25 g water in 100 g DM soil) for 100 days in an incubation chamber (Termaks B8420S, Norway, Bergen). Soil without SS material was incubated as control. The water level was maintained by regulating the weight up to 125 g twice a week. The field capacity of the soil was 30% but we chose to keep the humidity slightly lower to avoid anaerobic conditions, corresponding to 67% of field capacity. During the incubation experiment, the glass jars were covered by a plexi-glass with drilled holes to ensure constant humidity.

Total sample number for incubation at the start of the experiment (day zero), were 15 for each of the SS materials (powder and pellets). In addition, 3 samples (not incubated, control) with each material were stored directly at −18°C in plastic zipper bags. At increasing intervals at day 1, 14, 21, 69 and 100, three samples were taken out of the incubation chamber and stored similarly as above at −18°C. All these samples were analysed for mineral N according to NS-EN ISO 11885, after extracting 40 g frozen soil samples in 200 mL of 1 M KCl prior to analyses. During the incubation period, at day 0, 5, 15, 35, 72 and 100, the incubated glass jars were sealed for one hour by using a lid. Gas samples from all the remaining incubated glass jars each time (decreasing numbers) were taken by using vials crimp seal serum glass and a needle for gas samples through a silicone stopper in the lid. Gas samples were analysed by gas chromatography.

Statistics

The pot experiment had a complete 5 × 3 factorial design (five fertilisers incl. control, three temperatures). The data were analysed using two-way analysis (fertilisers, temperatures) followed by a one-way analysis for each temperature (ANOVA, GLM procedure). Analyses were performed by Minitab 16.1.0 (Microsoft, State College, PA, USA). Tukey multiple comparisons test were used for pairwise comparisons of treatments, with a setting of $\alpha = 0.05$.

In the incubation experiment, there were three samples (replicates) for each sampling date for mineral analyses, and a decreasing number of replicates (remaining samples) for each gas sampling date. The values from the measurements of mineral N and nitrous oxide emissions fluxes are presented as averages and standard deviations.

Results and discussion

Experimental SS pellet production

Based on initial testing it was not possible to extrude the SS powder without the addition of a lubricator and binder. The low-fat content created a high friction and heat, resulting in blocked extruder die holes. In addition, the low powder binding properties (low protein, high ash) gave poor pellet durability. The same results were also achieved during initial testing on a pellet mill. Wheat is considered as a first-choice starch-based binder in feed pellets (Thomas and van der Poel 1996; Ytrestøyl et al. 2015) and as a first approach, selected in this study. Soybean oil was selected as the lubricator. Both ingredients are easily accessible. The pellet had a diameter of 3.6 ± 0.1 mm, which is within the expected range for a fertiliser pellet.

The mechanical durability test simulates the forces applied during transportation and distribution (Thomas and van der Poel 1996). The pellet product had a durability of 94%. This may allow successful mechanical spreading in field, although not tested in this study. The SS powder had a high dust fraction (18% <44 µm) and low bulk density (317 g L^{-1}) and was therefore of limited relevance due to storage, transport and application challenges (generated a large amount of dust). The pellet product had a density of 467 g L^{-1}. This is lower than commercial organic and mineral fertilisers which have bulk densities in the range of about 650–1000 g L^{-1}. However, the high durability and a highly reduced dust problem made it more suitable for use as a fertiliser compared to the SS powder. The aim for the project was to document the possibility to produce a SS pellet fertiliser and economical optimisation was not the scope of this study. Extrusion processing is more costly than pelletising, and due to the downstream drying step, a wet process are costlier than a dry process. Nevertheless, suggested further work is to optimise the pellet production process for reduced processing costs and optimise the level and type of binder used. This can be other starches, molasses, lignosulfonates, clay, bone meal or fish silage. It is also important to further study the effect of processing and pellet physical properties on the dissolution rate and N-availability. Finally, a pellet product with a balanced nutrient ratio, by adding of K-rich resources, would make it more relevant for practical use (Brod et al. 2018).

Potato growth

The results clearly showed that SS is an effective potato fertiliser, thus with slightly lower tuber biomass than for MF at similar plant-available N-levels (Table 2). However, results showed a tendency of later plant emergence, fewer stems, later haulm growth cessation, and lower tuber numbers for the SS fertiliser than for MF. The delayed emergence and growth cessation are probably caused by a delayed N-availability from the organic SS fertiliser compared to MF. These growth chamber results are in accordance with results from previous field trials (Tor J. Johansen, unpublished).

Stem numbers did not seem to vary with levels of SS. However, there was a tendency of increasing tuber numbers per stem with increasing levels of SS fertiliser. This is difficult to explain, but is probably influenced by higher N- or P-availability (see Table 1) at higher fertiliser levels (Jenkins and Ali 2000). In general, tuber numbers and sizes are regulated by complex interacting mechanisms, and are determined by numbers of stems per plant, number of tubers per stem, and yield (Struik et al. 1990).

N uptake, N recovery efficiency and N availability

For all growth temperatures, the total N uptake at the various fertiliser treatments was highest for SS3, lowest for SS1 and intermediate for SS2 and MF, thereby following the ranking of applied N (Table 3). Interestingly, the total N uptake for MF and SS2 at all temperatures were approximately at the same levels, indicating similar N availability of the supplied 1 g MF and 1.38 g SS per pot.

NRE in this potato trial was around 90% for MF, and about 70% for the different levels of SS (Table 3). The high NRE for MF indicate a low level of N lost as gases (nitrous oxide, nitrogen dioxide, nitric oxide or ammonia), and a high level of N utilisation in the produce. Under field conditions, there is a potential risk for leakage as the N supplied as MF is available for the plants at application. However, in this experiment, leakage from pots is not relevant, and only N lost as gas (denitrification or ammonia volatilisation) or N bound in structural chemical compounds, may cause unavailability.

NRE is dependent on the N fertilisation amount, and are in general highest when the fertilisation rate is low. Thus, as the NRE is equal for all SS pellet treatments, it

Table 2. Average potato (*Solanum tuberosum*) growth data ($n = 6$) at emergence and harvest from a controlled climate trial with various fertilisers (F) and dosages at three temperatures (T). Pellets from shrimp shell powder in three N-levels (SS1-3; estimated plant-available N-dosages of 50, 100 and 150% of mineral fertiliser, MF) and no fertiliser (control, NF).

Temperatures and fertilisers	Emergence (days)	No. of stems per plant	Haulm maturity (% greenness)	Haulm DM (g per plant)	No. of tubers per plant	Tuber FM (g per plant)	Tuber DM[a] (%)
9°C (124 d)							
MF	19.5b	7.3a	10.8c	11.0b	30.8a	413a	23.2bc
SS1	24.2a	4.7b	40.8ab	6.1c	11.0bc	237b	23.9ab
SS2	23.3a	3.8b	46.7ab	10.5b	15.5b	363a	22.6bc
SS3	23.2a	4.8b	63.3ab	13.3a	14.3b	407a	21.7c
NF	24.5a	3.2b	20.0bc	0.8d	4.5c	51c	25.2a
15°C (82 d)							
MF	11.7	6.5a	21.7c	15.7b	14.3a	390a	23.5ab
SS1	12.5	4.2b	27.5bc	8.5c	8.7b	216c	24.6ab
SS2	13.7	4.0b	44.2b	16.4b	11.0ab	268b	23.7ab
SS3	13.3	3.8b	64.7a	28.0a	10.5ab	314b	22.2b
NF	15.3	2.8b	15.0c	1.2d	2.0c	59d	26.2a
21°C (68 d)							
MF	8.8b	5.8	9.2b	18.5b	18.2a	364a	22.2a
SS1	10.0a	5.5	19.2b	10.9c	7.5c	192c	22.7a
SS2	10.0a	4.2	26.7b	19.3b	13.3b	294b	22.3a
SS3	10.0a	4.7	60.0a	28.1a	17.5ab	294b	22.9a
NF	10.5a	3.2	10.0b	2.1d	2.5d	36d	23.6a
P-values (ANOVA)							
T	0.000	0.505	0.003	0.000	0.000	0.000	0.003
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000
$T \times F$	0.172	0.851	0.398	0.000	0.000	0.002	0.111

Notes: Growth periods (d) were different at the various growth temperatures.
Values within columns not having any lowercase letters in common are significantly different by Tukey's multiple comparisons test.
[a]Tuber dry matter (DM) measurements are based on merged tubers from two and two plants at each temperature ($n = 3$).

Table 3. N-application, N-uptake and N recovery efficiency (NRE) per plant in potato (*Solanum tuberosum*) for mineral fertiliser (MF) and three levels of shrimp shell (SS1-3). NF is control with no fertiliser. Average results ($n = 3$), ±SEM for NRE.

Temperatures and fertilisers	N-applied (g)	N-uptake Haulm (g)	N-uptake Tubers (g)	Total N-uptake (g)	NRE
9°C (124 d)					
MF	1.00	0.20c	0.73b	0.94b	0.83 ± 0.04
SS1	0.68	0.15c	0.48c	0.63c	0.77 ± 0.05
SS2	1.35	0.27b	0.74b	1.02b	0.67 ± 0.06
SS3	2.03	0.38a	1.03a	1.43a	0.65 ± 0.03
NF	0.00	0.01d	0.09d	0.11d	
15°C (82 d)					
MF	1.00	0.33c	0.68ab	1.03b	0.93 ± 0.02
SS1	0.68	0.18d	0.36c	0.55c	0.66 ± 0.03
SS2	1.35	0.49b	0.58b	1.08b	0.73 ± 0.02
SS3	2.03	0.76a	0.76a	1.53a	0.70 ± 0.04
NF	0.00	0.02e	0.08d	0.10d	
21°C (68 d)					
MF	1.00	0.29bc	0.75a	1.07b	0.94 ± 0.02
SS1	0.68	0.21cd	0.39b	0.61c	0.71 ± 0.09
SS2	1.35	0.42b	0.61a	1.06b	0.69 ± 0.02
SS3	2.03	0.82a	0.71a	1.56a	0.71 ± 0.02
NF	0.00	0.03d	0.08c	0.12d	
P-values (ANOVA)					
F		0.000	0.000	0.000	
T		0.000	0.000	0.145	
$F \times T$		0.000	0.002	0.386	

Notes: Growth periods (d) were different at the various growth temperatures. For each temperature, values for N application and uptake within columns not having any lowercase letters in common are significantly different by Tukey's multiple comparisons test.

indicates that the potato can use the entire available N from all three supplied amounts of SS pellets (approximately 70%, Table 3). The NRE after all SS fertilisation treatments was low compared to MF, which show that about 30% of added N is unavailable and bound in highly complex chemical structures, or lost as gases. The results matched N mineralisation for SS powder in the incubation experiment, but not for SS pellets (67% and 39%, respectively, Figure 1), which indicate better conditions for N mineralisation in the pot experiment than in the incubation experiment. These deviating results might be explained by different soil texture and moisture conditions, in accordance with studies by Jones et al. (2007) and Hayakawa et al. (2009).

SS powder has approximately the same chemical property as SS pellets, and the humidity, pH and temperature during incubation were equal for the two materials. The physical property is thereby the main difference between powder and pellets, and the main explaining factor for differences in mineral N amount after incubation. In the mineralisation process, the compact concentration of organic material in the pellets, might lead to higher microbial activity around and inside the pellets (anoxic hotspots), which favour denitrification instead of nitrification in the N cycle (Breland 1994; Cabrera et al. 1994a, 1994b; Petersen et al. 1996). This theory corresponds well with the denitrification fluxes for SS pellets and SS powder in our studies (Figure 2). Additional factors is that powder has a higher probability for dissolving, and a greater surface for microbial attack than pellets.

In conclusion, it is possible to produce a SS pellet product that can be used as a potato fertiliser (with potassium supplements), thus with a delayed N-availability compared to mineral fertilisers. The NRE of SS pellets was on average around 70% in the pot experiment, showing that about one-third of the added N as SS pellets might be unavailable for the potato plants during the growing season. The risk of N-loss trough N_2O emissions, demonstrate a need for further knowledge of potential denitrification for SS pellets under

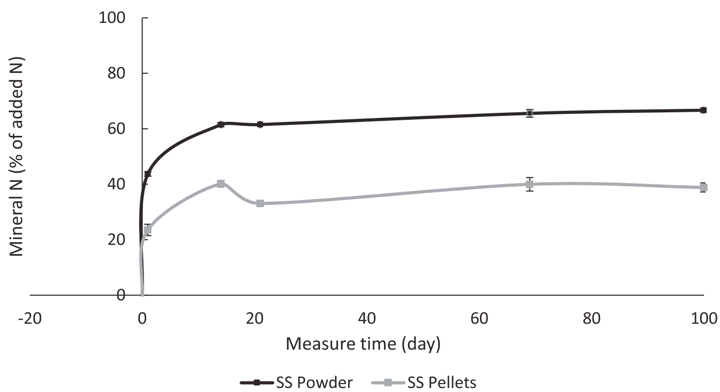

Figure 1. Mineral N (NO_3^- and NH_4^+) mineralised from shrimp shell (SS) pellets and SS powder during 100 days of incubation in soil at 15°C and constant humidity. Average results ($n = 3$) ± SD.

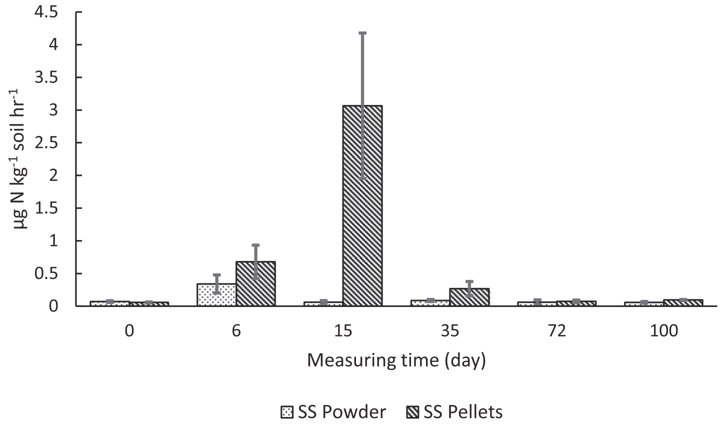

Figure 2. Nitrous oxide (N_2O) emissions from shrimp shell (SS) pellets and SS powder during 100 days of incubation in soil at 15°C and constant humidity. Average results (*n* varying) ± SD.

field conditions. For the practical relevance of the product, a more balanced nutrient ratio by adding K-rich material to the pellets would be advantageous.

Acknowledgements

This study is part of a project owned by the producers' organisation OTTAR (contact: Halgeir Jakobsen), with Stella Polaris AS (contact: Jaran Rauø) as project participant (Project no. 182724 in The Research Council of Norway). Thanks to a previous employee in NIBIO, Christian Uhlig, for contribution to planning of the experimental set-up. Dr. P. Dörsch (NMBU), helped with the analysis of N2O in the gas samples from the incubation experiment and interpretation of the results. We thank Prof. T.A. Breland, NMBU, for supervision of the incubation experiment.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Norges Forskningsråd [grant number 182724].

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ISBN: 978-82-575-7838-7
ISSN: 1894-6402

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