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Pathways to phase-out contentious inputs from organic agriculture in Europe

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SUMMARY:

In organic growing, both in Norway and elsewhere in Europe, significant amounts of fertiliser products not derived from certified organic farming are used, such as dried poultry manure or other types of fertilisers derived from conventional farms such as animal by-products. Organic agriculture aims to be independent of conventional agriculture, and fertilisers derived from harvesting of natural materials may be a relevant alternative. Catching of wild fish, and collection or cultivation of seaweeds, result in residual products which contain essential plant nutrients. Sediments of fish bones, which are residues from hydrolysis of fish remains used to produce fish oil and soluble proteins, are rich in nitrogen and phosphorus. Residues from seaweeds (algae fibre), after extraction of soluble nutrients sold as a liquid fertiliser, are rich in potassium and sulphur. However, we do not know much about how such residues affect plant growth. This topic was studied in a pot experiment with annual (Westerwold) ryegrass, which was harvested five times during the experimental period, April-August 2018. We had four replicates per treatment, and fish bones and algae fibre were applied either as fresh material or after drying at 105 °C. The control treatment was an experimental soil without any fertiliser, and we also had a treatment with calcium nitrate (Calcinit). The three types of fertilisers were applied in low and high amounts, where we aimed at a fertilisation level corresponding to 300 or 600 kg nitrogen (N) per ha (corresponding to 30 or 60 kg per daa). However, the actual amounts of N applied in fish bones, and algae fibre were somewhat lower than this. The total number of treatments was 11.

Application of fish bones gave a significant increase in the production of ryegrass. In total, across two N levels, and the drying status of materials, the accumulated above-ground yield over five harvests (stubble included) was 3.3 g DM per pot for fish bones, as compared with 2.2 g DM per pot in the control treatment. Fertilisation of ryegrass with algae fibre and Calcinit both gave an average yield of 2.6 g DM per pot. Converting these numbers to kg DM per ha, the accumulated yields were 7 166 kg/ha without any fertiliser, 8 541 kg/ha with algae fibre, 8 616 kg/ha with Calcinit and 10 916 kg/ha with fish bones. The average yield increase in % of the control yield was 19% with algae fibre, 20% with Calcinit and 52% for fish bones.

Nitrogen was the nutrient which was supposed to give the most significant effect on the production of plant dry matter. Somewhat more N was applied with Calcinit than with fish bones, but the yields were still considerably higher with fish bones. This was likely explained by the phosphorus (P) content of the fish bones (no P is present in Calcinit). Drying at 105 °C did not reduce the positive growth effect since slightly higher yields were observed with dried than with fresh materials. Fertilisation with algae fibre led to luxury uptake of potassium (K) in above-ground material of ryegrass while causing low uptake of calcium (Ca) and magnesium (Mg). In spite of high concentrations of arsenic (As) (33 mg/kg DM) in algae fibre, the concentrations of As in ryegrass plants amended with this material were below the limit of detection.

Both fish bones and algae fibre contain valuable plant nutrients and organic matter, which may have a positive effect as fertilisers and soil amendments. However, they are not well balanced as compared with the requirements of agricultural crop plants. Hence, unless special nutrients are requested, they need to be combined or mixed with other sources of nutrients and organic matter, to produce a valuable fertiliser which will also be easy to use in practice.

Several further studies are required to possibly develop commercial fertiliser products for organic growing from marine-derived residual materials such as fish bones and algae fibre.

Sammendrag

I økologisk dyrking, både i Norge og i resten av Europa, brukes det betydelige mengder av konvensjonell fjørfegjødsel og andre gjødselprodukter basert på konvensjonelt husdyrhold. Økologisk landbruk ønsker å være uavhengig av konvensjonell drift, og gjødsel basert på materialer som høstes fra naturen kan være et aktuelt alternativ. Tradisjonelt havfiske, og innsamling eller dyrking av brunalger, gir restprodukter som inneholder viktige plantenæringsstoff. Sedimenter av fiskebein, etter at kvernet fiskeavfall er hydrolysert til olje og løselig protein, inneholder mye nitrogen og fosfor. Rester etter pressing av plantestyrkende algeekstrakt fra grisetang (algefiber) inneholder mye kalium og svovel. Men hvordan virker slike produkter på plantevekst? Det undersøkte vi i et potteforsøk med ettårig (Westerwold) raigras, som ble høstet fem ganger i forsøksperioden, som varte fra april til august 2018. Vi hadde fire gjentak av hver behandling, og tilførte fiskebein og algefiber enten i fersk form, eller etter tørking ved 105 °C i varmeskap. Som kontroll hadde vi forsøksjord uten gjødsel, og forsøksjord tilsatt kalksalpeter (kalsiumnitrat). De tre gjødseltypene ble tilført i to ulike mengder, slik at vi totalt kom opp i 11 behandlinger. Vi tok sikte på å tilføre gjødsel tilsvarende 300 eller 600 kg nitrogen (N) per ha (30 eller 60 kg per daa). For algefiber og fiskebein viste det seg imidlertid at tilført mengde var noe lavere enn dette.

Gjødsling av raigras med fiskebein ga rask og stor plantevekst, med en total produksjon av tørrstoff (TS) (blad, stengler og stubb, ikke røtter) over fem høstinger på 3.3 g per potte, som et gjennomsnitt for begge N-nivå og ferskt og tørket materiale. I jord uten gjødsel var 2.2 g TS per potte. Gjødsling med algefiber og kalksalpeter (Calcinit) ga i gjennomsnitt samme avlingsnivå, 2.6 g TS per potte. Omgjort til kg per daa blir de akkumulerte avlingene 717 kg/daa uten gjødsling, 854 kg med algefiber, 862 med kalksalpeter og 1092 med fiskebein. Gjennomsnittlige avlingsøkning, når økningen beregnes som prosent av kontrollavling, var 19% for algefiber, 20% for kalksalpeter og 52% for fiskebein.

Vi forventet at tilførselen av nitrogen (N) ville være det som ga størst utslag på produksjonen av plantemateriale. Til tross for at mer N ble tilført med kalksalpeter enn med fiskebein, var tørrstoffavlingen høyest i pottene med fiskebein. Dette skyldes sannsynligvis at fiskebeina, i motsetning til kalksalpeter, inneholder mye fosfor (P).

Tørking ved 105 °C reduserte ikke den positive veksteffekten av fiskebein eller algefiber, for avlingene var minst like høye ved tilførsel av tørka som av ferskt materiale. Gjødsling med algefiber førte til luksusopptak av kalium (K) i raigraset og lavt opptak av Ca og magnesium (Mg). Til tross for høye konsentrasjoner av arsen (As) (33 mg / kg TS) i algefiber, var konsentrasjonen av As i raigrasplanter med tilførsel av algefiber under deteksjonsgrensen.

Både fiskebein og algefiber inneholder verdifulle plantenæringsstoffer og organisk materiale som kan ha en positiv effekt som gjødsel og jordforbedring. Imidlertid er ikke næringsinnholdet godt balansert med hensyn til behovene til jordbruksplanter. Med mindre det er ensidige gjødselslag som etterspørres, bør algefiber og fiskebein bør kombineres med hverandre og/eller med andre

kilder for å bli et godt balansert gjødselprodukt. Det trengs grundigere studier på mange områder for å utvikle kommersielle gjødselprodukter fra marine restmaterialer som fiskebein og algefiber.

COUNTRY:	Norway
COUNTY:	Møre og Romsdal
MUNICIPALITY:	Tingvoll
LOKALITET:	Tingvoll

APPROVED	**PROJECT LEADER**
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Preface

Organic agriculture seeks to utilise locally available resources of plant nutrients and organic matter, and to be independent of non-organic agriculture, e.g. with respect to inputs of fertilisers. Currently, significant amounts of poultry manure and other animal-derived fertilisers from non-organic management are used in organic farming in Norway, as well as in many other European countries. One option to reduce the input of fertilisers derived from non-organic agriculture could be to harvest nutrients and organic matter from the sea and use these materials for fertilisation. Since significant amounts of nutrients are leached and lost from terrestrial environments into the sea, this could close nutrient gaps and reduce current challenges of eutrophication, which are substantial, for example, in the Baltic Sea. Both fish and seaweed (algae) have a long tradition to be used as soil amendments. However, to potentially replace, e.g. pelletized poultry manure as a commercial product by a fertiliser made from marine materials, a range of issues need clarification. For instance, crop plants need a fertiliser which is balanced into macro- and micronutrients. A commercial fertiliser product must be easy to store and spread, as well as biologically stable, and should not contain pathogens or toxic compounds. The county council of Møre and Romsdal has provided funding for a research project, “Marine rest raw materials for fertilisers to organic agriculture” (RESTOR), which is run by NORSØK during 2018 - 2020 to explore such issues.

After communicating with industry stakeholders, we decided to start-up with two materials: seaweed residues, and fishbone sediments from hydrolysed fish residues. Both materials are available in the region of Møre and Romsdal, and elsewhere along the coastline in significant quantities. The algae fibre used in the study and reported here was a left-over product from the production of seaweed extracts, applied for promoting plant growth. Fish residues are usually treated by formic acid for conservation during hydrolysis; however, the fish bones used in this study were remains from pilot-scale hydrolysis with no use of formic acid. Seaweed residues contain high amounts of potassium (K) and some sulphur (S), and fish bones contain high amounts of nitrogen (N) and phosphorus (P). If applied in combination, these marine residues may give a well-balanced fertiliser for crop plants. However, in this study, we applied the materials separately, to study the growth effect of each material.

A pot experiment was conducted where relevant marine materials were applied as fertilisers to annual (Westerwold) ryegrass. Using marine residues for fertilisation is of interest also for other European countries. NORSØK participates in the Horizon 2020-project “**Pathways to phase-out contentious inputs from organic agriculture in Europe**” (Organic-PLUS), where marine-derived fertilisers may be one option to reduce the contentious input of non-organic animal manure to organic farming. Whereas the RESTOR-project provided resources for conducting the pot experiment, the Organic-PLUS project has provided funding for chemical analyses and the writing of this report.

We would like to acknowledge our project partners Eva Salomon, Research Institutes of Sweden (RISE); Jannicke F. Remme, and Andreas Austnes, SINTEF Ocean AS; Egidijus Dauksas, NTNU Ålesund; Tron Kjønno, Bogestilla AS; Hege Gjerde, Algea AS; Annelise Chapman, TANGO Seaweed AS; Amund Pedersen, Fjordlaks AS; Bjørn Stabbetorp, Felleskjøpet Agri AS, and Kristine Marie Hestetun, Norwegian Food Safety Authority, for their kind assistance. The fish bones used in the pot experiment were provided by Jannicke F. Remme, SINTEF Ocean, and algae fibre by Hege Gjerde, Algea AS. Peggy Haugnes and Susanne Friis Pedersen, NORSØK, and Anne de Boer, Norwegian Institute for Bioeconomy Research (NIBIO, Tingvoll) are kindly acknowledged for providing help with the experiment. We would also like to thank Anita Land, Kirsty Mckinnon, Martha Ebbesvik, Solveig Johnsen and Rosann Johanssen, NORSØK, Torfinn Torp (NIBIO, Ås), and Linde Melby for sharing their knowledge and help with diverse things to produce this report.

The photos used to make pictures presented in this report are taken by Ishita Ahuja.

Tingvoll, 06.09.19

Ishita Ahuja

Innhold

1 Introduction

Nutrients present in various organic waste possess the potential to replace traditional fertilisers in agriculture. Fertiliser products may be made from processed organic waste from agriculture, fisheries and aquaculture, households, recreational areas, and the food processing industry. Compound products such as park waste compost and digestates from anaerobic digestion of various organic waste are gradually becoming more familiar for Norwegian farmers and growers (Brod et al., 2017; Brod et al., 2018). In other countries, where anaerobic digestion has a longer history than Norway, such fertiliser products have become quite common to use (Mostafazadeh-Fard et al., 2019; Tampio et al., 2016).

Norway has a significant marine sector with great potential to recycle organic material and plant nutrients present in residues from algae and the fish industry as fertilisers. Marine residues may perform well as fertilisers (Arioli et al., 2015; Hartz et al., 2010; Illera-Vives et al., 2015a; Illera-Vives et al., 2015b; Nabti et al., 2017). Several fertilisers made from fish waste and/or seaweed have been produced and tested on crop and horticultural plants (Archer et al., 2001; Craigie, 2011; Illera-Vives et al., 2017; López-Mosquera et al., 2011; Sahu et al., 2014). A lot of commercial products made from fish processing waste and/or seaweeds are available mainly in the US and Canada, partly also approved for use in certified organic growing (OMRI, 2019a, b; Sharma et al., 2014).

In Norway, in recent years, the cultivation of macroalgae (in Norwegian: tang, and tare) has gained attraction due to its potential role in the emerging bioeconomy, with applications in human food, domestic animal and fish feed products, fertiliser, prebiotics, cosmetics, bioactive peptides, pharmaceuticals and nutraceuticals (Stévant et al., 2017; Thangstad, 2018). However, macroalgae may contain trace elements with possible negative consequences for public health, such as arsenic (As) (Maher, 1984; Stévant et al., 2018a; Stévant et al., 2018b). Contrarily, they may possess valuable trace elements such as iodine (I).

Coastal communities have applied seaweeds traditionally as a soil amendment and fertiliser (Rebours et al., 2014a; Rebours et al., 2014b). It is over 65 years since the first commercial seaweed extract was manufactured for agricultural use in 1951, as described by Craigie (2011). Seaweed-derived extracts/fertilisers are commercially available worldwide (Chojnacka, 2012; Nabti et al., 2017; Sharma et al., 2014), and some are also approved by the Organic Materials Review Institute (OMRI) for use in certified organic crop production (OMRI, 2019b). OMRI is an international non-profit organisation that determines which input products are allowed for use in organic production and processing https://www.omri.org/.

Seaweeds have been exploited through ages for fodder and as fertiliser along the Norwegian coast but lost their value through modernisation of agriculture and due to the production of mineral fertilisers (Holm, 2002). In his book about Norwegian geography, "*Norges Naturlige Historie"* from 1752, bishop Pontoppidan described the use of seaweed (tang) as fertiliser and for soil improvement (Indergaard, 2010). Norwegian farmers have been using algae for soil improvement by gathering seaweed from the shoreline and spreading it over their fields (Fleddum, 2013). In the field experiments near Trondheim, during 1958 – 1960, the seaweeds were applied to turnips, fodder beets, and cauliflower. Seaweed fertilisers showed positive effects in addition to those expected from the content of N, P and K (Myklestad, 1963). On a study tour to Jæren, Norway, in 2000,

McKinnon and Holm experienced that farmers still used seaweeds as fertiliser and to reduce soil erosion (McKinnon et al., 2004). In trials conducted by NORSØK (McKinnon et al., 2004), the seaweed knotted wrack (*Ascophyllum nodosum*) composted with straw, wood cuttings and peat, and freshly cut knotted wrack, both increased yields of oilseed rape, and subsequent yields of ryegrass.

While the fertilisation effects of fresh or decomposing seaweeds are well-known and studied, residual materials from industrial processing of seaweed are also available, for instance, after extraction of nutrients as liquid fertiliser products, or production of alginate. These residues contain valuable nutrients and may affect positively on soil characteristics such as water holding capacity (Riley, 2002). In a field-study with potatoes (Riley, 2002), algae fibre by-product (with about 40 % perlite) was obtained from FMC Biopolymer, a large producer of alginate. The application enhanced soil concentrations of sodium (Riley, 2002). However, it increased the water holding capacity of various test soils and showed a positive effect on yields of potato tubers. The growth effect of 1 tonne algal fibre (DM) per daa, containing about 12 kg N, was equal to 20 - 25 kg of compound fertiliser NPK (11-5-17). Significant amounts of mineral N were present in the soil at potato harvest, and some of this N was available for a subsequent crop of ryegrass in the next growing season (Riley, 2002).

In the pot experiment, presented here, the algae fibre was obtained from the Algea AS in Kristiansund. The algae fibre is residual material from knotted wrack collected along the coast of Norway, which has been dried, grounded and extracted by acid/alkali to produce fertiliser extracts, which are exported and marketed by Valagro AS, which owns the company Algea AS.

Large amounts of fish processing waste are produced in countries with a significant fish industry, such as Canada, US, UK, India, the Republic of Korea, China, Senegal, Spain and Norway (Balraj et al., 2014; Ghaly et al., 2013; Kazemi et al., 2017; Teh and Sumaila, 2013). Through the discussions with partners involved in the RESTOR project, we came to know that grinded fish bones, conserved by formic acid, are a type of fish waste that is currently poorly utilised, and usually disposed of by incineration. The conserved fish bones are produced when fish residues, mainly from filleting of cod and saithe such as bones, heads, skin, fins, and intestines, are grinded, mixed with formic acid and stored in large tanks as fish silage. During storage, top layers of oil and soluble protein separate by hydrolysis. On the bottom, there is a layer of sediments, comprised mostly of bone residues, but also other particles such as cartilage. The sediments are rich in nutrients and hence were of interest for further study as fertilisers. The high contents of P and N in fish bones (Toppe et al., 2007; Zheng et al., 2013) makes them very relevant to be used for fertilisation purposes.

Annual ryegrass (*Lolium westerwoldicum*) is a common forage grass in Norwegian agriculture. This species is also commonly used as an experimental crop, due to its ability to germinate very fast and rapidly produce a new canopy after repetitive cutting. Hence, the pattern of nutrient uptake can be recorded in detail with several harvests of the canopy.

The current study was conducted to evaluate the effects of fish bones and algae fibre, applied as fertilisers on the growth and nutrient uptake of ryegrass, and soil nutrient concentrations. These residues were selected because they are readily available in the county of Møre and Romsdal. We aimed to answer the following questions:

(i) Whether fish bones and algae fibre enhance the growth and nutrient uptake of ryegrass?

(ii) Whether the addition of fish bones or algae fibre to soil has modified the soil characteristics such as pH, loss on ignition and nutrient concentrations?

(iii) Whether the soil that was fertilised with fish bones and algae fibre contained potentially toxic trace elements, for example, arsenic, lead, and mercury?

2 Materials and methods

2.1 Soil, fertilisers and other experimental conditions

Sifted topsoil (0 - 20 cm) from a field called “Sagmyra” (translation: the moorland (“myr”), next to the sawmill (“sag”), on Tingvoll farm (location of NORSØK) was used. This soil (loamy sand; “siltig mellomsand”) has about 70% sand, 23% silt, and 7% clay in the mineral fraction. It contains 12% organic matter and about 40 mg AL-extractable phosphorus (P) per kg air-dry soil which is low to medium-high. Extraction by ammonium-acetate lactate (AL) is the standard method to characterise soil P availability in Norway. The soil has a pH (H_2O) of about 5.5. This soil was used because it had proven good characteristics to demonstrate the effect of various organic fertilisers in previous studies (Løes, 2017). With medium to low P concentration, effects of fertilisation are easier to explore. Further, the soil structure remains satisfactory over time due to a high content of sand and organic matter. The volume weight of the soil, measured as bulk density in field condition (Løes et al., 2013) was 1 kg per dm^3, and the factors used for converting fertilisation per pot to fertilisation per ha of topsoil (0 - 20 cm) was 2000 kg of soil per ha (1 kg/dm^3 x 0.2 m x 10 000 m^2), and 600 g of soil per pot (550 g plus 50 g to cover ryegrass seeds).

Fertilisation levels are usually higher in pot experiments than in the field. Ryegrass is a crop which can take high nitrogen (N) fertilisation, and we aimed for an application of 300 or 600 kg N per ha, corresponding to 0.09 or 0.18 g N per pot (600 g soil). We applied the fertilisers at the start of the experiment only.

Soil, or soil mixed with fertilisers, was filled into plastic pots of 12 cm height with aeration holes in the bottom, where a round piece of the metallic net was placed to avoid soil loss. The pots were filled carefully with the requested weight of soil, which was distributed in three portions. Between each portion, the pot was gently kicked towards the table 10 times, while turning it a quarter around for each kick. This was done to ensure even physical conditions for the growth of plants.

To establish an appropriate level of watering the pots, the pot “field capacity” was measured by filling five pots with 600 g of sifted soil as described above, carefully saturating them with water and then let them drain for about 1 hour until no more free water was visible under the pot. The water content of the soil upon filling was 26%, implying that 600 g of soil contained 444 g of dry soil. On an average, this amount of soil could hold 349 g of water, which means that at field capacity (in this sifted soil), when 600 g is filled into a plastic pot, the water content is about 44% and the pot contains 444 g soil + 349 g water = 793 g. For watering in pot experiments, it is usually recommended to apply water to 50 - 70 % of field capacity, which implies that each pot should weigh between 618 (50%) and 688 (70%) after watering. Pots with soil were placed in trays containing a water-absorbing cloth and 11 pots. The weight of the tray, including cloth and 11 empty pots was 1010 g. Hence, the recommended weight per tray after watering would be between 7808 g (= 618 g x 11 + 1010 g), and 8578 g (= 688 g x 11 + 1010 g). The weight of the tray at field capacity would be 8723 + 1010 g = 9733 g.

2.1.1 Fish bones as fertiliser

Frozen fish bones received from pilot-scale hydrolysis with no use of formic acid (Picture 1) were obtained from SINTEF Ocean AS Ålesund in February 2018. The average content of DM in fish bones, as determined by four replicate samples of 50 g, after drying at 105 °C was 52.5%.

Picture 1. Materials used as fertilisers in the pot experiment with ryegrass.

The content of N in fish bones was estimated following (Toppe et al., 2007), where the fish bones from cod and saithe (caught in Norway), were reported to contain on average 356 g protein per kg of lipid-free DM, which corresponds to 57.28 g N kg^{-1} DM (6.25% total N in protein). As per calculations, following this paper, we applied 3.0 and 6.0 g fresh fish bones, and 1.5 and 3.0 g dried fish bones per pot, corresponding to fertilisation levels of 308.5 kg N and 617.0 kg N ha^{-1} (Table 1). The subsequent

Table 1. An overview of treatments in the pot experiment.

	Treatment	Details	Sample codes
1	Control	Experimental soil (ES): 600 g/pot	ES
2	Dried fish bones (low N) 0.052 g N/pot = 262 kg N/ha	ES: 600 g/pot + Dried fish bones (DFB): 1.5 g /pot	ES+DFB-LN
3	Dried fish bones (high N) 0.104 g N/pot = 524 kg N/ha	ES: 600 g/pot + DFB: 3.0 g/pot	ES+DFB-HN
4	Fresh fish bones (low N) 0.052 g N/pot = 262 kg N/ha	ES: 600 g/pot + Fresh fish bones (FFB): 3.0 g/pot (52.5% DM)	ES+FFB-LN
5	Fresh fish bones (high N) 0.104 g N/pot = 524 kg N/ha	ES: 600 g/pot + FFB: 6.0 g/pot (52.5% DM)	ES+FFB-HN
6	Dried algal fibre (low N) 0.057 g N/pot = 190 kg N/ha	ES: 600 g/pot + Dried algal fibre (DAF): 4 g/pot	ES+DAF-LN
‘7	Dried algal fibre (high N) 0.114 g N/pot = 380 kg N/ha	ES: 600 g/pot + DAF: 8 g/pot	ES+DAF-HN
8	Fresh algal fibre (low N) 0.057 g N/pot = 190 kg N/ha	ES: 600 g/pot + Fresh algal fibre (FAF): 12.8 g/pot (32 % DM)	ES+FAF-LN
9	Fresh algal fibre (high N) 0.114 g N/pot = 380 kg N/ha	ES: 600 g/pot + FAF: 25.6 g/pot (32 % DM)	ES+FAF-HN
10	Mineral fertiliser (low N) 0.09 g N/pot = 300 kg N/ha	ES: 600 g/pot + Calcinit (CAL): 0.580 g/pot	ES+CAL-LN
11	Mineral fertiliser (high N) 0.18 g N/pot = 600 kg N/ha	ES: 600 g/pot + CAL: 1.161 g/pot	ES+CAL-HN

chemical analysis of fish bones (Eurofins, Jena), showed that 1.5 and 3.0 g amounts of dried fish bones per pot, corresponded to fertilisation levels of 262.0 kg N or 524.0 kg N ha^{-1} (Table 1). The amounts of N shown for algae fibre and fish bones in Table 1 were calculated from the analytical values of samples dried at 105 °C (Table 4).

The dried bones used in the experiment were dried at 105 °C, with no active aeration but an opening for letting out the humid air. We assumed that drying at high temperature could reduce the N content of the material, and this was the reason to compare dried and fresh (frozen) fish bones. However, we did not consider that drying at 30 °C would affect the N content significantly. Therefore, the fresh fish bones were dried at 30 °C before sending the material for chemical analysis, in a cabinet with active aeration.

In early 2019, another batch of sediments of fish bones, which were received from a commercial fish industry was sent for chemical analyses to Eurofins. The analyses showed that the method of drying of fish bone sediments significantly affects the N content. Nitrogen is easily lost, even at a temperature of 30 °C, if the drying is combined with active aeration. When fish bone sediments from two batches (ICP tanks) were analysed after passive drying at room temperature with the laboratory, Eurofins (about 25 °C), the total N contents were 13.3 and 15.3% of DM. However, the samples that were dried at 40 °C with active aeration from a fan contained only 5.0 and 5.9% of total N. Samples that were dried at 105 °C with no active aeration had 4% of total N.

The results of chemical analysis of fish bones used in the present study are presented in section 3.1.

2.1.2 Algae fibre as fertiliser

Fresh algae fibre (Picture 1) was obtained from Algea AS, Kristiansund on March 22, 2018. In 2002, Algea became part of the international company Valagro AS. The company collects and processes knotted wrack (*Ascophyllum nodosum*) to make extracts and phytocomplexes for use in agriculture and animal feed (Algea, 2019). The production of seaweed extracts from *A. nodosum* leaves a filter cake (algae fibre), which is currently used as a substrate for composting organic waste in a nearby municipality (Vestnes). However, new regulations for fertilisers and soil amendments made from organic waste were proposed in 2018, with a strict limit to arsenic (NorwegianAgricultureAgency, 2018). If these regulations are adopted, such kind of utilisation of this material would be hampered. The average content of DM in algae fibre, as determined by four replicate samples of 150 g fresh weight, after drying at 105 °C, was 32%.

The content of N in the algae fibre was estimated using analytical values from former chemical analyses. We had access to two analyses of algae fibre from Algea AS in 2011 (Table 2, samples 2 and 3), and one from a former Norwegian study (Hanssen, 1996; Table 2, sample 1). We used the average N value of samples from 2011 to calculate the amount of N in algae fibre.

Assuming an average value of 1.55% of DM of total N (Table 2), we applied amounts of 12.8 g and 25.6 g of fresh algae fibre per pot, or 4.0 g and 8.0 g of dried algae fibre per pot, corresponding to 300 kg or 600 kg N ha^{-1}. The subsequent analysis of dried algae fibre (Eurofins, Jena), showed that the applied amounts corresponded to fertilisation levels of 190.0 or 380.0 kg N ha^{-1} (Table 1). The dried algae fibre used in the experiment was dried at 105 °C, with no active aeration but an opening for letting out the humid air. The dried algae fibre samples, which became very compact after drying,

were grinded in a mortar before applying the material to the soil (Picture 1), and before sending it to Eurofins for analysis. Fresh algae fibre was relatively easy to mix with the soil.

Table 2. Chemical composition of algal fibre from Algea AS: samples from 2011 and 1995.

Macronutrients, carbon and pH

Sample	DM (%)	Total-N g /100g DM	Total-C g/100g DM	NH4-N mg/kg DM	NO3-N mg/kg DM	P g/kg DM	K g/kg DM	Ca g/kg DM	Mg g/kg DM	S g/kg DM	pH
1 *	81.3	1.0	27.8	7.4	9.3	1.8	7.2	28.3	-	6.6	8.9
2	73	1.4	-	6.4	1993	1.1	5.3	31	8.6	6.9	9.1
3	70	1.7	-	1.2	2968	0.8	58	31	9.2	7.0	9.2
Mean of 2 & 3	71.5	1.55									

Micronutrients and potentially toxic elements

Sample	Na g/kg DM	B g/kg DM	Cl g/kg DM	Fe mg/kg DM	Cd mg/kg DM	Hg mg/kg DM	Pb mg/kg DM	Ni mg/kg DM	Cr mg/kg DM	Zn, mg/kg DM	As, mg/kg DM
1	37	-	7.4	544	0.96	0.012	0.28	-	-	-	-
2	41	43	-	230	0.58	0.007	1.1	1.5	0.98	61	21
3	8.5	0.05	-	120	0.64	0.018	1.1	1.3	0.56	76	22

The data presented in this table were made available from Algea AS as a part of a former cooperation activity with Dr Sissel Hansen, NORSØK.
***Sample 1:** Received from Algea in Drammen, Norway; analysed at «Landbrukets analysesenter», Ås 16.3.1995 (Hanssen, 1996). **Sample 2:** Sampled by Algea AS, Kristiansund 2.12.2011; analysed by Eurofins. **Sample 3:** Sampled by Algea AS, Kristiansund 6.12.2011; analysed by Eurofins.*

2.1.3 Mineral fertiliser

Calcium nitrate was purchased as Calcinit from YaraLiva. Calcinit has 15.5% N + 19% Ca (Picture 1). It is a free-flowing fully water-soluble nitrogen and calcium fertiliser. One gram of Calcinit contained 0.155 g N. Hence, 0.58 g Calcinit was applied to supply the soil with 0.09 g N per pot, corresponding to 300 kg N ha^{-1}, and 1.16 g to supply the soil with 0.18 g N per pot, corresponding to 600 kg N ha^{-1} (Table 1).

2.1.4 Mixing of soil and fertilisers

Each fertiliser was thoroughly mixed into a portion of the experimental soil before the soil was filled into the pots, on March 27, 2018, as described in section 2.1.

2.2 Design of the pot experiment

The experimental set up was a randomised "block" design with soil as control (no fertilisation), and ten treatments of soil plus fertilisers fish bones (dry and fresh), algal fibre (dry and fresh), and Calcinit, all in two N levels (Table 1). One replicate pot from each treatment (n = 11) was put together on a tray (= "block"), which was covered by a thick textile cloth to ensure even distribution of applied water. The plastic pots were labelled with codes indicating the treatments (Table 1, Picture 2). When all pots were filled and distributed into trays, 35 ml of deionised water was applied on top of each pot to moisten the soil surface.

Thirty seeds of annual ryegrass (*Lolium westerwoldicum*) (Barenbrug grass seeds) were then evenly placed on the soil surface and covered with 50 g of finely sieved soil. About 1050 g of deionised water was then applied to the cloth of each tray, to achieve a weight of 8300 g per tray, to let the soil moisten from below. The pots were then covered with a plastic sheet until the seeds germinated, which occurred on April 4, 2018 (Picture 2).

Picture 2. Pots were labelled, filled with soil or soil mixed with fertilisers, kept on trays, covered with plastic sheets until germination of ryegrass seeds, and kept on tables in a growth room with controlled environmental conditions.

The analysis of fish bones and algae fibre (Eurofins, Jena) (described later in section 3.1), after drying at 105 °C and 30 °C showed different values for total N (Table 4). Since the values were not very much affected by the difference in temperature, the calculation of N amounts given in fresh and dry material (Table 1) is based on the N concentration after drying at 105 °C. For algae fibre, the total N-concentrations after drying at 105 and 30 °C were 1.43 and 1.51 g 100 g^{-1} DM. For fish bones, the total N-concentrations after drying at 105 °C and 30 °C were 5.24 and 5.04 g 100 g^{-1} DM. If we had instead used the value obtained after drying at 30 °C, the applied amount of total-N would be slightly different than presented in Table 1; 402.6 as compared with 380.0 kg N/ha for algae fibre, and 504.0 as compared with 524.0 kg N/ha for fish bones.

We did not analyse fresh fish bones and fresh algae fibre since we were not aware of the significant impact that drying conditions may have on N-concentrations.

2.3 Watering and management of ryegrass plants

At the beginning of the experiment (Picture 3), plants were given more water than intended. The weight of a tray was 9800 g after watering (Figures 1 and 2), which is slightly above the field capacity of 9733 g (section 2.1). Since pots with different fertilisation were mixed in the same tray and should not affect each other via leaching, this should have been avoided; however, even if the soil was quite wet, free water did not occur on the soil surface. Watering was levelled off, and from 13.04.2018, and until the end of the experiment (08.08.2018) and happened thrice a week (Monday-Wednesday-Friday). At each occasion, the initial weight of each tray was recorded, and after applying deionised water to the underlying cloth the weight of trays with pots was brought up to 8500 g (Mondays and Wednesday), and 8700 g on Fridays (Figures 1 and 2). During each watering procedure, all trays were turned horizontally 180°, and their position on the table was systematically changed (inner tray became outer tray, and others were moved one position inwards), to equalise differences in growing conditions. Along with the ryegrass, some weeds also germinated, which were picked out from the soil by forceps and left to decompose on the soil surface of the pot.

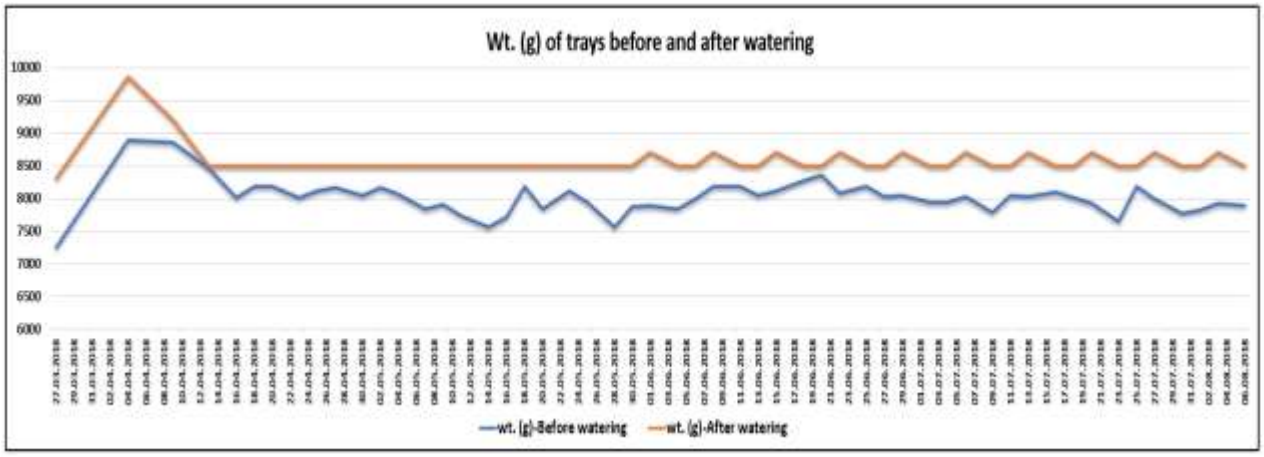


Figure 1. Watering during the experimental period (27.03.2018 - 06.08.2018).

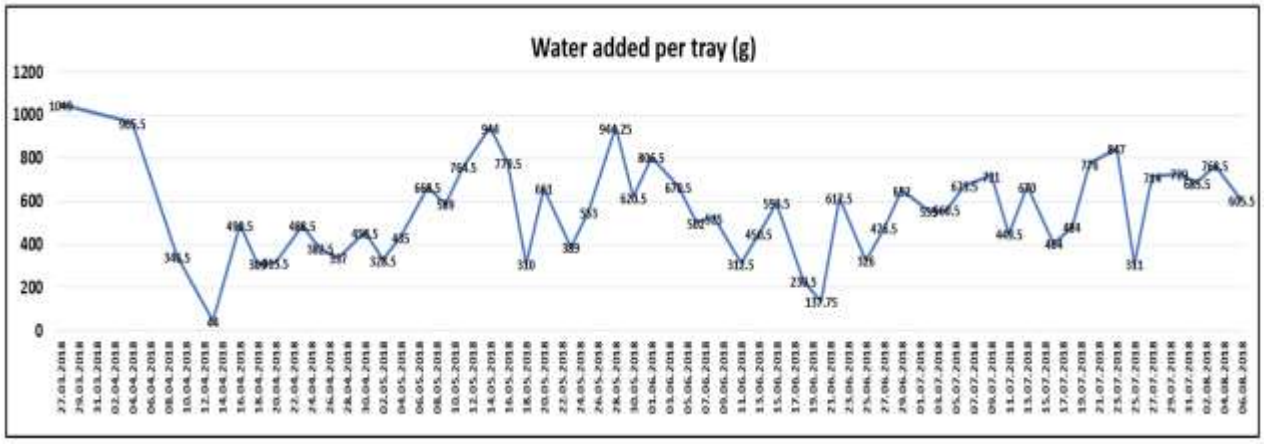
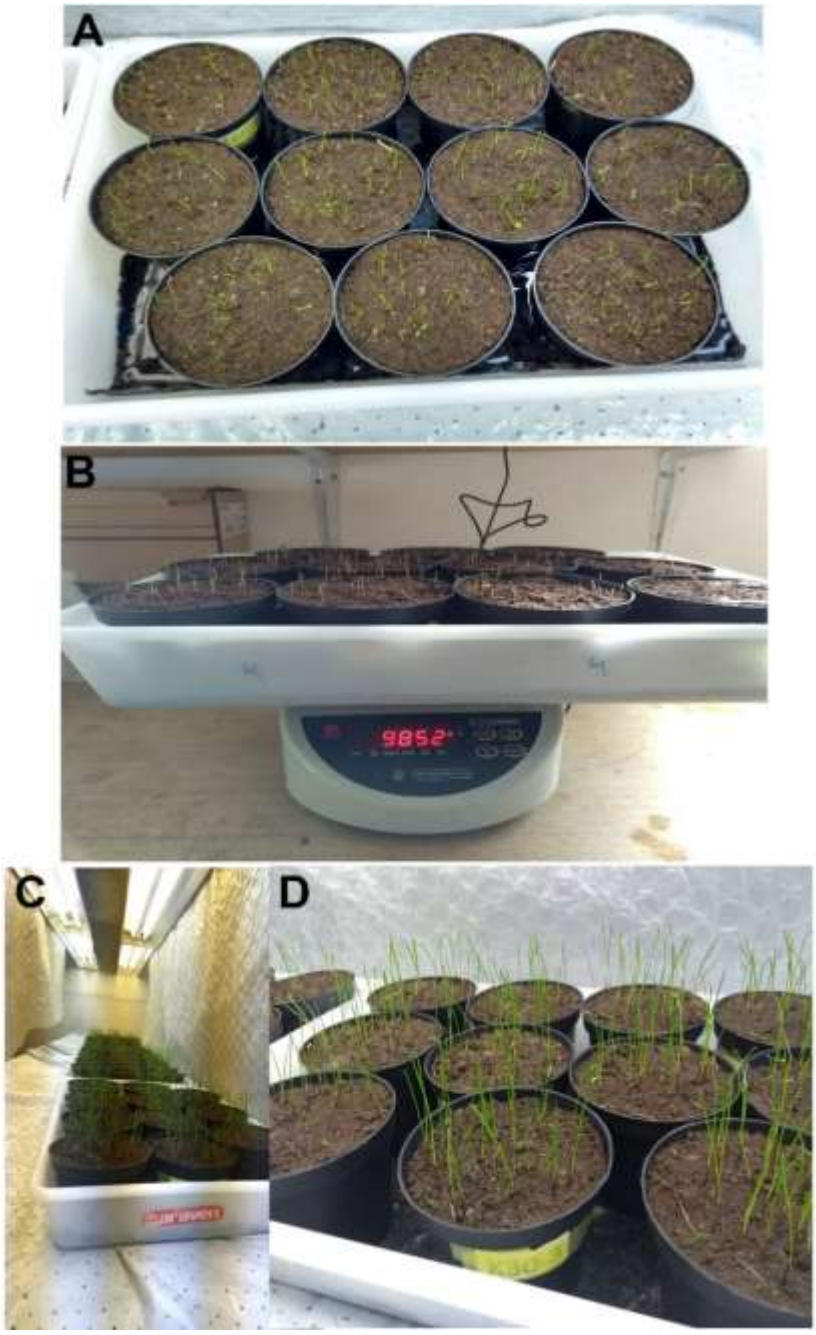


Figure 2. Amount of water that was added per tray on Monday, Wednesday and Friday during the experimental period (27.03.2018 - 06.08.2018).



Picture 3. Seedling emergence of ryegrass as observed on April 4, 2018. (A and B) Weighing of the tray with 11 pots to add water up to the required weight. (C, D) Plants as observed on 6th April 2018.

2.4 Harvesting of ryegrass plants

A total of five harvests were completed during the entire growth period of 125 days (04. 04.2018-08.08.2018) (Picture 3, Table 3). The four first harvests were performed by cutting the above-ground plant material 4 cm above the soil surface, and the weight was recorded per pot. At the final harvest, this procedure was repeated, but then the stubble of the plants was also cut, at the soil surface, and weighed separately. All aboveground plant material which was harvested was put in paper bags. The plant material was weighed (fresh wt.) and dried at 40 °C to a constant weight, and the dry weights were recorded.

Table 3. Main activities during the pot experiment.

Date		Activity
27.03.2018		Filling of pots with soil/soil + fertilisers, weighing and watering of trays.
28.03.2018		Placement of seeds on the soil surface of each pot and covering of pots with plastic sheets.
04.04.2018		Seeds germinated. Plastic sheets were removed. The trays with pots were weighed and watered.
06.04.2018 26.04.2018	–	Most of the weeds were found before the first harvest, were removed and placed on the soil surface of the same pot.
27.03.2018 06.08.2018	–	Watering of trays with 11 pots/tray during the whole experimental period (Figures 1 and 2).
26.04.2018		First harvest. Recording of fresh and dry weights of above-ground plant material, stubble height 4 cm.
16.05.2018		Second harvest. Recording of fresh and dry weights of above-ground plant material, stubble height 4 cm.
13.06.2018		Third harvest. Recording of fresh and dry weights of above-ground plant material, stubble height 4 cm.
11.07.2018		Fourth harvest. Recording of fresh and dry weights of above-ground plant material, stubble height 4 cm.
08.08.2018		Fifth and final harvest and completion of the experiment. Weighing of each pot; recording of fresh and dry weights of above-ground plant material, stubble height 4 cm; besides, the weight of stubble at height 0 cm. The soil in pots was loosened, and soil samples were taken for chemical analysis (one sample per pot).

2.5 Microscopic examination of soil

After the final harvest of plants, soil samples were observed under a stereomicroscope (Leica M205C; Leica Microsystems, Switzerland; Camera model: MC170 HD); to look for indications of remains or possibly degradation of fish bones and algal fibre. The soil samples (about 5 g) from unfertilised and fertilised soil (high N level) were placed in glass Petri dishes and subsamples brought to a glass plate, to take images. The images were made using the software Leica Application Suite version 4.4.0.

2.6 Chemical analysis of organic fertilisers

Representative samples of fish bones and algal fibre were dried at 30 °C and 105 °C and sent to Eurofins, Moss, Norway, with further analysis at Eurofins Umwelt Ost GmbH, Jena, Germany. Upon communication with the laboratory, samples were subject to an analysis package adapted to agricultural compost (Eurofins Agro, 2019), which included the analyses of dry matter content, bulk density, loss on ignition (LOI), pH, total-N, ammonium-N, nitrate-N, total organic C, AL-extractable nutrients, Olsen-extractable P, total P, K, Ca, Mg, S (Table 4), micronutrients and potentially toxic elements (Table 5). The concentrations of total K, Ca, and Mg were recorded upon request.

In the laboratory, the samples were put in flat plastic bowls and stored in a drying room, where samples of compost etc. normally dry within about 15 hours. The average drying temperature is about 25 °C ± 5 °C, which is a bit lower than in a normal drying process. Instead of heating the room, water is extracted using the circulating air. That process is more efficient at lower temperatures. After drying, samples were grinded with a razor blade mill and sieved to < 1.5 mm particle size. The grinded samples were then aliquoted for further processes.

For determination of the content of dry matter, ashes (loss on ignition) and density, fresh (non-dried) material from the samples was used. For dry matter, 100 g of fresh material was dried at 105 °C.

The bulk density was determined by filling a graduated cylinder with the material, and a specific weight put on top to compress the material for 3 minutes. After that, the bulk density was calculated from records of volume and weight.

For determination of loss on ignition (LOI), an aliquot of fresh material was pre-dried at 40 °C. After that, the sample was grinded with a razor blade mill, sieved to < 1.5 mm and ignited at 450 °C. The LOI method for the determination of organic matter involves the heated destruction of all organic matter in the sample (Øien and Krogstad, 1989).

The pH in the materials was measured after mixing the grinded samples with de-ionised water (1:2.5 v/v). The settling time is between 1 and 3 hours, and pH is measured in the liquid phase of the suspension.

The total N was determined by modified DIN EN 13654 method**,** Soil improvers and growing media - Determination of nitrogen - Part 1: Modified Kjeldahl method. The Kjeldahl-N is a well-known method to determine total-N in organic materials, which implies to heat the material with H_2SO_4 to release N as $(NH_4)_2SO_4$ and measure the amount of N indirectly by titrating the amount of NH_4 in boric acid (Brod et al., 2018; EN13654-1, 2001; Øien and Krogstad, 1989).

Ammonium-N (NH4-N) was determined following standard EN ISO 11732 (Brod et al., 2017; ENISO11732, 1997; Magnusson et al., 2008). The dried and grinded samples were extracted with a mixture of $CaCl_2$ and DTPA (diethylenetriaminepentaacetic acid or pentetic acid), called CAT extract. After shaking and filtration, concentrations of ions were detected by a photometrical method where complex building chemicals were added to the filtrate to produce coloured components to be detected in a continuous flow analysis (CFA).

The total organic carbon (TOC) was determined by combusting the dried and grinded samples and detecting the carbon via elemental analysis.

AL-extractable P, K, Ca, Mg and Na were determined following the AL-method (Egner et al., 1960), where the soil is extracted with 0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75 with a ratio of soil to solution of 1:20 (w/v) (Riley et al., 2003). In Norway, this method has been used since 1960, and this method is also used in Sweden, Iceland, Germany, and the Netherlands (Krogstad, 2009). The method is described in a standard as SS 0283110 + T1 (Standardising Commission in Sweden, 1993).

Easily extractable P was determined by the “Olsen-P” method, which estimates the relative bioavailability of ortho-phosphate (PO_4-P) in soils or other substrates by extraction with alkaline sodium bicarbonate (pH 8.5) solution. The P concentration in the extract is determined calorimetrically. It is applicable to soils that are mildly acidic to alkaline pH and is based on the method developed by (Olsen et al., 1954) to correlate crop response to fertiliser on calcareous soils.

For determination of the concentrations of “total” P, K, Ca, Mg, micronutrients and heavy metals, aqua regia (a mixture of nitric acid, HNO_3 and hydrochloric acid, HCl) was used for extraction. For an accurate determination of total concentrations of elements, the addition of hydrofluoric acid (HF) to the aqua regia is required. This is rarely done with commercial laboratories, and aqua regia-extracted concentrations are hence often stated as “so-called totals”. Detection of the elements was done by ICP-MS (inductively coupled plasma – mass spectrometry). Sulphur and micronutrients (S, B, Co, Fe, Mn, Mo, Cu, Zn), and potentially toxic metals (Ni, Cd, Pb, As and Hg) were determined by EN ISO

11885 method (ENISO11885, 2009; ISO11885, 2007). Since we did not expect that the drying temperature would affect the concentrations of minerals except nitrogen, total K, Mg and Ca, plus micronutrients and potentially toxic metals were only analysed in samples dried at 30 °C.

2.7 Chemical analysis of soil

After the final harvest, the soil in each pot was carefully emptied into a tray, roots gently removed, and samples of soil for chemical analysis were collected from each pot and were dried overnight at 30 °C. In addition to the 44 samples from experimental pots, four parallel samples were taken before the study started to characterise the initial experimental soil. The soil samples were sent for chemical analysis at Eurofins Umwelt Ost GmbH, Jena, Germany (Eurofins, 2019). The package "Soil Analysis" provided information about soil volume weight, soil pH (soil: water suspension 1:5 w/w) (EN ISO 10390)(ISO10390:2005), loss on ignition (LOI), and AL-extractable nutrients (P, K, Mg, Ca, and Na), (see section 2.6)(SS 028310 + T1)(Standardising Commission in Sweden, 1993). Further, the concentrations of potentially toxic elements As, Cd, Cr, Cu, Ni, Pb and Zn were analysed according to the standard NS-EN ISO 17294-2, and Hg according to EN 16175-1. One soil sample amended with Calcinit was lost in the Eurofins laboratory.

As per personal communication from Eurofins, based on method (ISO10390:2005), the laboratory at Jena is measuring pH in H_2O, and not in $CaCl_2$. ISO 10390:2005 *specifies an instrumental method for the routine determination of pH using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H_2O), in 1 mol/l potassium chloride solution (pH in KCl) or in 0,01 mol/l calcium chloride solution (pH in $CaCl_2$)* (ISO10390:2005). As per Krogstad and Haraldsen, the method (EN ISO 10390) that Eurofins applies to measure pH, is based on the addition of 50 ml deionised water to 10 ml dry soil to get 1:5 (volume fraction*)* (Krogstad and Haraldsen, 2018). However, in Norway, the standard method to measure pH in the soil is using 10 ml dry soil in 25 ml de-ionised water (Krogstad, 2009; Krogstad and Haraldsen, 2018; Riley et al., 2003). In principle, the method that Eurofins applies would dilute the soil solution, and it would affect the concentration of hydrogen ions. As mentioned by Krogstad and Haraldsen, a comparison of these two methods showed that between pH 5.5 -and 7, the difference between the two methods is neglectable (or small). Outside this interval, however, the use of a higher proportion of water as in EN ISO 10390 can affect the pH value by 0.2 to 0.3 (Krogstad and Haraldsen, 2018).

2.8 Chemical analysis of above-ground plant material

The dried above-ground plant material from all harvests, including the stubble, was compiled for each replicate pot and sent for milling and chemical analysis to the Activation Laboratories (Actlabs, Canada), (Actlabs Agriculture, 2019). Actlabs is a commercial laboratory that is accredited to both ISO 17025 with CAN-P-1579 for specific registered tests (Actlabs, 2019; Laiho et al., 2012). The nutrients were analysed by the Plant Tissue Analytical Package (PTP), which includes N, P, K, Mg, Ca, Na, S, Fe, Al, Mn, B, Cu, and Zn. Additionally, As, Cd, Co, Cr, Cu, Pb, Hg, Mo and Ni were analysed by a Heavy Metal Package. Macro and micronutrients and potentially toxic elements were extracted in an open vessel by a combined nitric acid/peroxide digestion, using an in-house proprietary method. Total N was analysed by the combustion method (AOAC 990.03) (AOAC Official Method 990.03), while P, S, K, Mg, Ca, Na, Fe, B, Cu, Mn and Zn were analysed by inductively coupled plasma

optical emission spectrometry (ICP-OES). The minerals Mo, Se, Co, Al, Ni, Cr, As, Cd, Hg and Pb were analysed by inductively coupled plasma mass spectrometry (ICP-MS).

The contents of macronutrients, N, P, K, S, Mg, and Ca are given as % of DM, while the contents of micronutrients and potentially toxic elements (Fe, B, Cu, Mn, Zn, Mo, Se, Co, Al, Ni, Cr, As, Cd, Hg and Pb) are given as ppm.

The plant uptake of nutrients (mg per plant) was calculated by converting the concentrations of nutrients from % and ppm into mg g^{-1} DM and then multiplying with the amount of total plant total above-ground DM (foliage + stubble, g).

2.9 Evaluation of N fertilisation effects

The apparent nitrogen recovery (ANR, %) in total aboveground DM yield (stubble included) was calculated based on the difference method, following (Brod et al., 2017).

ANR (%) = N uptake (N+) – N uptake (NoN) / N applied x 100

Where: N uptake (N+) (mg N per pot) is N taken up in aboveground DM yield by fertilised plants;

N uptake (NoN) (mg N per pot) is N taken up in aboveground DM yield by non-fertilised plants (experimental soil), and

N applied (mg N pot) is N applied with the fertiliser.

The value shows how much of the applied N was utilised to produce above-ground plant tissue.

2.10 Statistical analysis

Even if the replicate plots were distributed in four trays acting as “blocks”, these “blocks” were moved systematically around to level out differences in environmental conditions. Hence, the tray (“block”) was not used as a factor in the statistical analysis. The data were analysed using one-way ANOVA (analysis of variance) for each type of fertiliser, to compare relevant results (e.g. total above-ground DM yield) with results obtained in the control treatment (no fertiliser). When the ANOVA showed a significant difference ($P < 0.05$), the statistically significant differences between treatments were evaluated by Tukey’s t-test. The relations between the application of different levels of nitrogen and above-ground DM accumulation were analysed by performing Simple Linear Regression by selecting option fitted line plot, where DM yield was response and nitrogen, applied in fertiliser, a continuous predictor. The statistical software that was applied for the analysis was MINITAB v.18.

3 Results and Discussion

3.1 Characteristics and chemical composition of fish bones and algae fibre

Samples of fish bones and algae fibre were dried at 30 °C and 105 °C. The values of pH, total organic carbon (TOC), C:N ratio, total- K, Mg and S were higher in algae fibre than in fish bones (Table 4). The TOC was almost two-fold higher in fish bones than algae fibre. The concentrations of total N, total P, P-Olsen, and total calcium (Ca) were higher in fish bones than algae fibre. The concentrations of P-AL, K-AL, Mg-AL, Ca-AL, and Na-AL were above the limit of detection in both materials.

Table 4. Characteristics and chemical composition of fish bones and algae fibre after drying at 30 °C and 105 °C.

Parameter	Algae fibre (dried at 105 °C)	Algae fibre (dried at 30 °C)	Fish bones (dried at 105 °C)	Fish bones (dried at 30 °C)
pH	10	9.6	6.8	6.9
TOC (g 100 g^{-1} DM)	32.3	31.3	16.2	15.1
C/N	23	21	3	3
DM (g 100 g^{-1})	98.7	91.4	99.5	93.4
LOI (g 100 g^{-1} DM)	54.9	54.7	33.6	33.5
Bulk density (g/l)	573	676	562	538
Total N (g 100 g^{-1} DM)	1.43	1.51	5.24	5.04
NH_4-N (g 100 g^{-1} DM)	0.01	0.006	0.015	0.037
NO_3-N (g 100 g^{-1} DM)	0.17	0.23	< 0.0005	< 0.0005
P-Olsen (mg 100 g^{-1})	69.7	49.9	421	472
P-total (g kg^{-1} DM)	2.4	2.5	120.0	120.0
Total Ca (g kg^{-1} DM)		68.0		300.0
Total K (g kg^{-1} DM)		130.0		1.7
Total Mg (g kg^{-1} DM)		25.0		3.8
S (g kg^{-1} DM)		15.0		2.8
P-AL (mg 100 g^{-1})	> 40	> 40	> 40	> 40
K-AL (mg 100 g^{-1})	> 40	> 40	> 40	> 40
Mg-AL (mg kg^{-1} DM)	> 60	> 60	> 60	> 60
Ca-AL (mg 100 g^{-1})	> 200	> 200	> 200	> 200
Na-AL (mg 100 g^{-1})	> 20	> 20	> 20	> 20

AL ammonium lactate; (AL-method), C:N carbon-nitrogen ratio, Ca Calcium, DM dry matter, N total nitrogen (Kjeldahl N), LOI loss on ignition, Na sodium, NH_4-N ammonium-nitrogen, NO_3-N nitrate- nitrogen, P phosphorous, P-Olsen (Olsen method), K potassium, Mg magnesium, S sulfur, TOC total organic carbon.

We had expected that a higher drying temperature would decrease the concentration of total N. We did not have much information about this at initiation of this pot experiment, but after the completion of that study, fish bones conserved with formic acid were received from a commercial hydrolysis process with Fjordlaks AS in two International Container Pool (ICP) containers. These fish bones were analysed for total N after three different drying procedures. The samples were taken from each ICP-container: 1) as frozen, 2) dried at 40 °C (active ventilation), or 3) dried at 105 C° (passive ventilation) and sent to Eurofins. Frozen bones were dried at the Eurofins laboratory in room temperature with de-humified air, as described in section 2.6.

The drying conditions significantly affected total-N values. Frozen bones, dried in air temperature, had 13 - 15 % total N (% of DM), whereas dried bones (also dried again at Eurofins) had 4 - 6% total N. This shows that drying may affect N concentration in fish bones. Possibly, for the samples of fish bones analysed from the pot experiment, the active ventilation used at 30 °C may have compensated for the effect of higher temperature, but passive ventilation at 105 °C, since the N concentrations were quite equal at both temperatures (Table 4). However, since the fresh bones used in the pot experiment were not dried at all, the growth effect of fresh bones should have been higher if more N was available in that material. The relationship between drying conditions and N concentrations in fish bones needs to be studied in more detail.

The concentrations of cobalt (Co) and molybdenum (Mo) were below the limit of detection (Table 5). Concentrations of boron (B), iron (Fe), and manganese (Mn) were higher in algae fibre than fish bones. Algae fibre had 5-fold higher levels of S than fish bones (Table 4), and a significant concentration of Fe and Mn (Table 5). For Cu and Zn, concentrations were about equal in algae fibre and fish bones. Zn concentrations were quite high in both materials (Table 6), close to the limit of class 0 soil amendment products. Soil conditioners in class 0 may be applied according to crop demands on all types of land (Table 6), whereas soil conditioners in class I may be used in amounts up to 40 tons of DW per ha of agricultural land over a period of 10 years, or applied as a top layer up to 5 cm on land not used for growing of food or feed crops. Soil conditioners in class II may be used in amounts up to 20 tons of DW per ha of agricultural land over a period of 10 years or applied as a top layer as described for class I products. Soil conditioners in class III may be used as a top layer as described for class I and II or used as a top layer up to 15 cm to cover waste deposits.

Table 5. Concentrations of micronutrients and potentially toxic elements (mg/kg DM) in fish bones and algae fibre after drying at 30 °C.

	Algae fibre	Fish Bones
B	94.0	27.0
Co	< 1.0	< 1.0
Fe	210.0	< 10
Mn	50.0	14.0
Mo	< 2.0	< 2.0
Cu	9.4	7.3
Zn	94.0	100.0
Ni	< 1.5	< 1.5
Cd	0.9	< 0.10
Pb	< 0.30	< 0.30
Hg	0.080	0.089
Cr	3.8	< 0.30
As	33.0	1.3

As Arsenic, B Boron, Cd Cadmium, Co Cobalt, Cr Chromium, Cu Copper, Fe Iron, Hg Mercury, Ni Nickel, Pb Lead, Mn Manganese, Mo Molybdenum, Zn Zinc.

The concentrations of potentially toxic elements were below the limit of detection for Ni and Pb in both materials (Table 5). Concentrations of Cd were 0.9 mg/kg DM in algae fibre, while they were below the limit of detection in fish bones. The concentrations of Hg were almost equal in both materials, whereas Cr and As were found in much higher concentrations in algae fibre than in fish bones. The concentration of As (Table 5) would hamper a future use of algae fibre as an ingredient in growing media or composts if the proposed new regulations are implemented (Norwegian Agriculture Agency, 2018). In the proposal for revised fertiliser regulations, a threshold of 5 mg As per kg of DW is proposed for soil conditioners or fertilisers classified in class 0. Further proposed threshold values for As are 8 mg in class I; 16 mg in class II and 32 in class III. For substrates to be included in soil conditioners and fertilisers, the values should not exceed class I threshold (8 mg) for class 0 products. For class I and II products of soil conditioners/fertilisers, the substrates should not contain more than the threshold for class II, and substrates used for a class III product may not contain more than the threshold value for class III products. For clarification, the proposed values for As have been shown in Table 6.

The concentration of Cr is well below the limit of 50 mg per kg DM for class 0 (Table 6), whereas for Cd, the concentration in algae fibre would categorise this material as a class II product.

Table 6. Limits of concentrations of potentially toxic elements (heavy metals, mg/kg DM) currently allowed in soil conditioners and organic fertilisers in Norway (LOVDATA 2003), and proposed limits for arsenic (Norwegian Agriculture Agency 2018).

Quality classes:	***0***	***I***	***II***	***III***
Cd	0.4	0.8	2	5
Pb	40	60	80	200
Hg	0.2	0.6	3	5
Ni	20	30	50	80
Zn	150	400	800	1500
Cu	50	150	650	1000
Cr	50	60	100	150
As	5	8	16	32

As arsenic, Cd Cadmium, Cr Chromium, Cu Copper, Hg Mercury, Ni Nickel, Pb Lead, Zn Zinc.

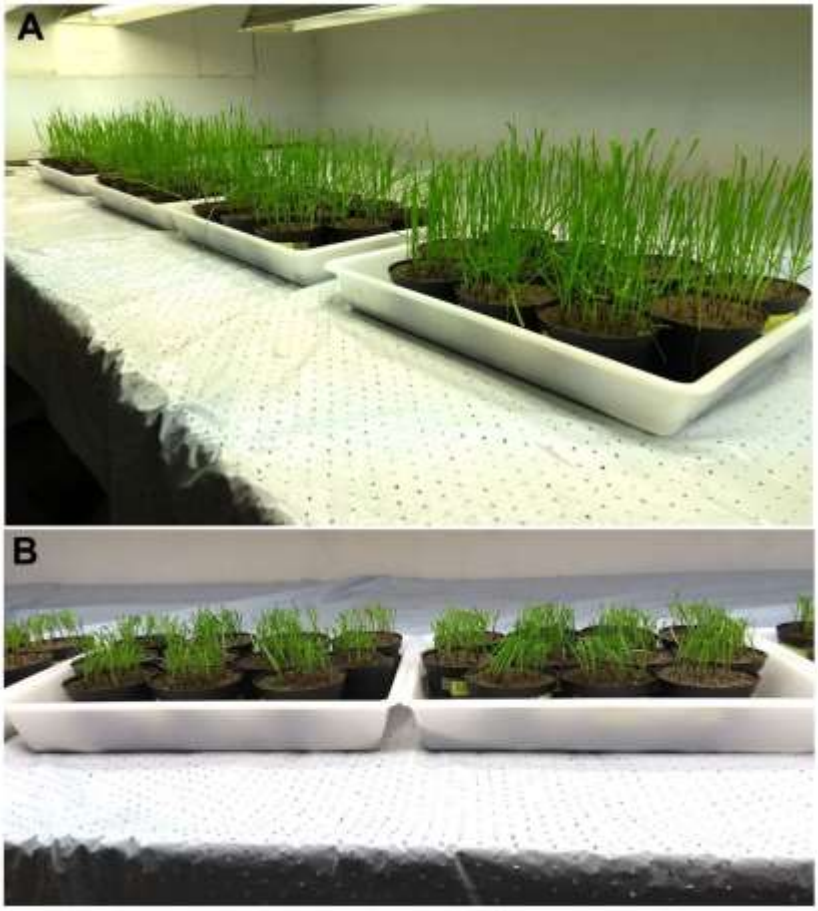
3.2 Germination and plant number at final harvest

In each pot, 30 ryegrass seeds were planted. Fertilisation did not affect germination as about 90% germination was observed in non-fertilised as well as fertilised pots (data not shown).

The number of plants per pot was recorded during the final harvest. No significant differences were observed for the average number of plants per pot, which varied between 24 (dried algae fibre, high N) and 29 (Calcinit, high N).

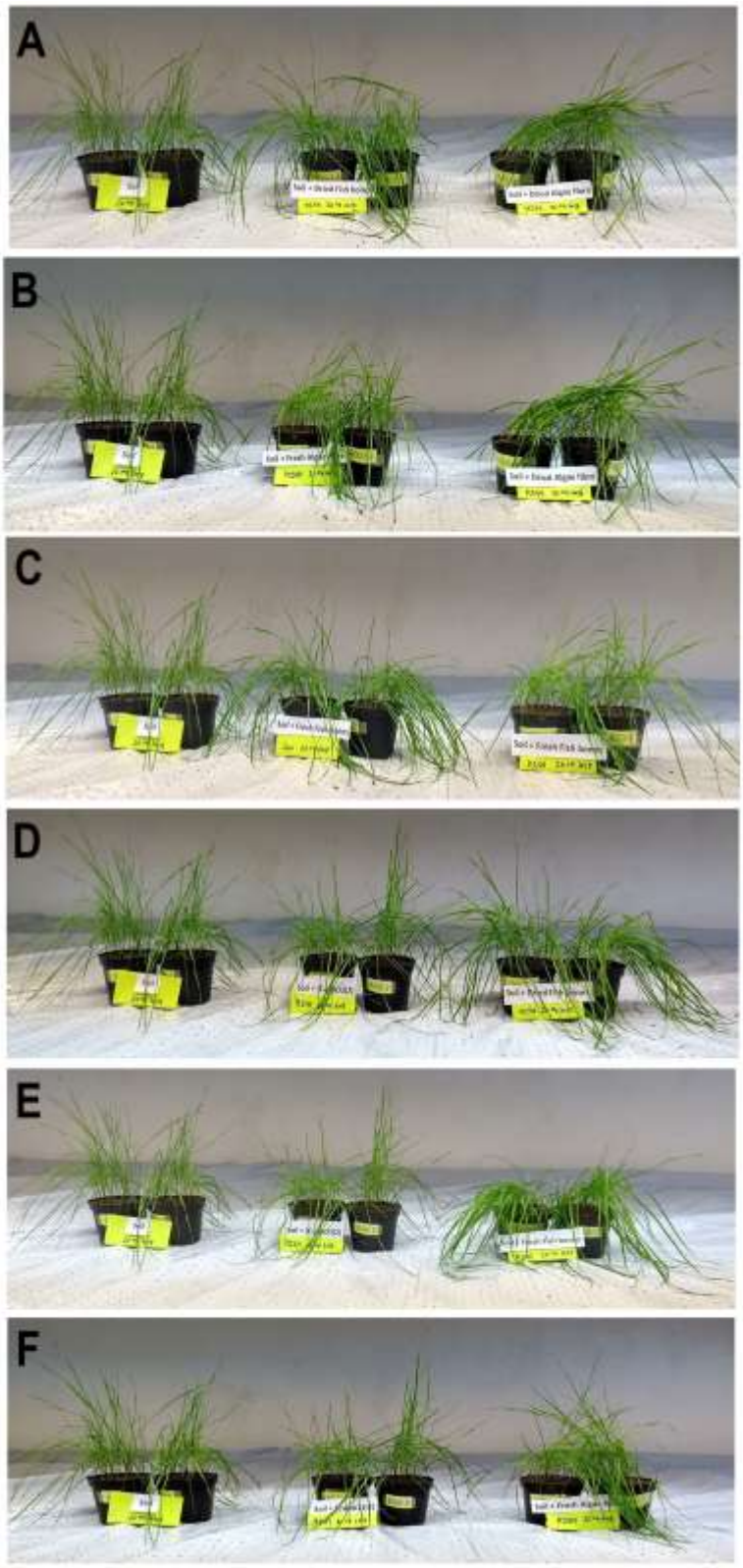
3.3 Effect of fertilisers on growth of ryegrass

3.3.1 The appearance of ryegrass plants at first harvest



Picture 4. The appearance of ryegrass plants on 26.04.2018 (first harvest), before harvest (A), and after harvest, with a stubble height of 4 cm (B).

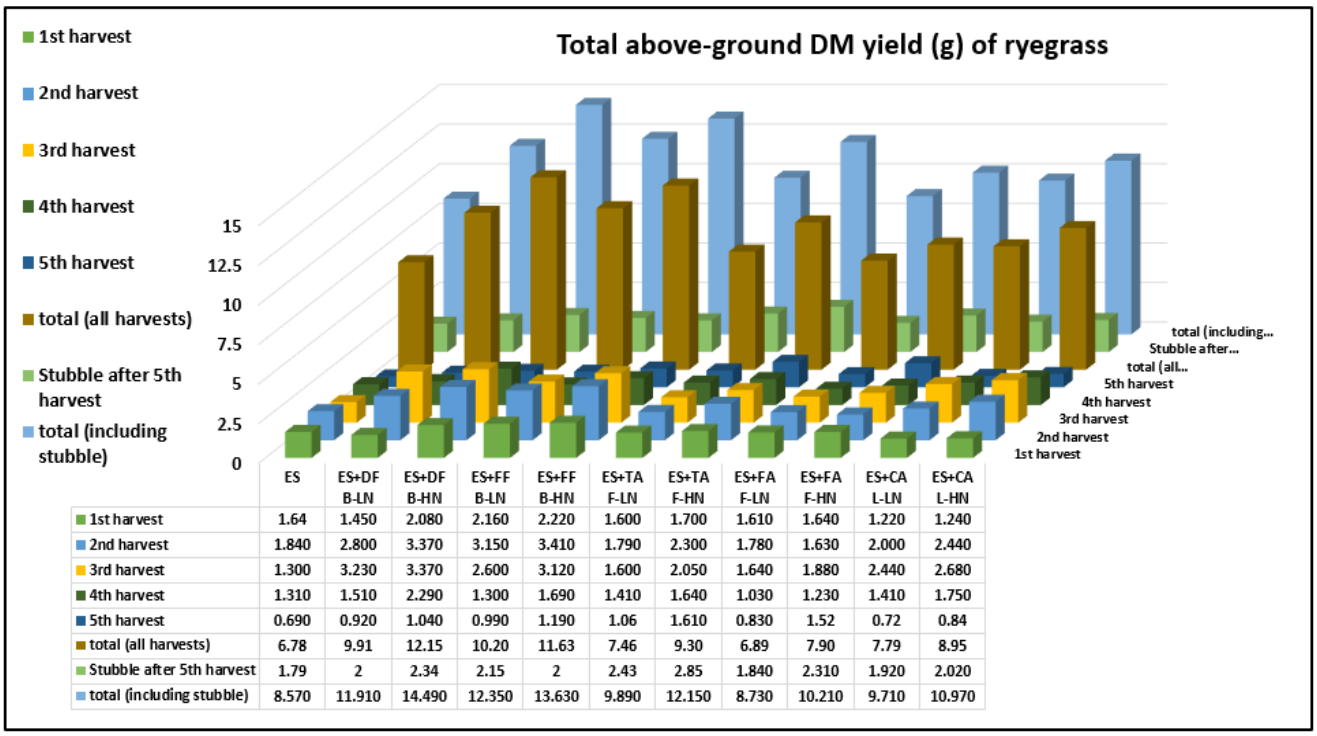
Pictures 4 and 5 show the appearance of ryegrass plants at, and after the first harvest. Plants that were given a high level of N showed a higher production of above-ground plant material for all treatments (Picture 5). Plants receiving high levels of N as fish bones produced more leaves at first harvest than those receiving Calcinit or no fertiliser (Picture 5D, E), and plants receiving high amounts of fresh algal fibre produced more leaves than those receiving Calcinit or no fertiliser (Picture 5 F).



Picture 5. Appearance and comparison of ryegrass plants on 26.04.2018 (first harvest), grown in soil (unfertilised), always to the left and in soil with three types of fertilisers, as explained in Table 1.
***(A)** ES, ES+DFB-HN, ES+DAF-HN **(B)** ES, ES+FAF-HN, ES+DAF-HN **(C)** ES, ES+FFB-LN, ES+FFB-HN **(D)** ES, ES+CAL-HN, ES+DFB-HN **(E)** ES, ES+CAL-HN, ES+FFB-HN **(F)** ES, ES+CAL-HN, ES+FAF-HN.*
ES= Experimental soil, FB= fish bones, AF= algae fibre, D= dried (material), F = fresh (material), HN= high N level, LN = low N level.

3.3.2 Yields of above-ground plant material

In Figure 3, the total above-ground production of plant material (dry matter, DM), *as a sum of the material produced in each of the four replicate pots in each treatment*, is shown. One reason to present the data in this way is that this shows the actual amounts of accumulated plant material that was produced during the study; is in total was 122.61 g.



	ES	ES+DF B-LN	ES+DF B-HN	ES+FF B-LN	ES+FF B-HN	ES+TA F-LN	ES+TA F-HN	ES+FA F-LN	ES+FA F-HN	ES+CA L-LN	ES+CA L-HN
1st harvest	1.64	1.450	2.080	2.160	2.220	1.600	1.700	1.610	1.640	1.220	1.240
2nd harvest	1.840	2.800	3.370	3.150	3.410	1.790	2.300	1.780	1.630	2.000	2.440
3rd harvest	1.300	3.230	3.370	2.600	3.120	1.600	2.050	1.640	1.880	2.440	2.680
4th harvest	1.310	1.510	2.290	1.300	1.690	1.410	1.640	1.030	1.230	1.410	1.750
5th harvest	0.690	0.920	1.040	0.990	1.190	1.06	1.610	0.830	1.52	0.72	0.84
total (all harvests)	6.78	9.91	12.15	10.20	11.63	7.46	9.30	6.89	7.90	7.79	8.95
Stubble after 5th harvest	1.79	2	2.34	2.15	2	2.43	2.85	1.840	2.310	1.920	2.020
total (including stubble)	8.570	11.910	14.490	12.350	13.630	9.890	12.150	8.730	10.210	9.710	10.970

Figure 3. Effect of different treatments on total above-ground DM yields of ryegrass from different harvests (n = 4).
ES= Experimental soil, FB= fish bones, AF= algae fibre, D= dried (material), F = fresh (material), HN= high N level, LN = low N level.

Overall, fertilisation with fish bones, algae fibre and Calcinit enhanced the above-ground DM yield compared to the experimental soil (Figure 3). The total above-ground DM yield (including stubble) showed the highest value (14.5 g) for plants that received high levels of N (524 kg N/ha) as dried fish bones. The lowest total yield was lowest (8.6 g = 2.2 g per pot) was found for plants that received no fertilisation. Even if the amount of N applied with Calcinit was higher than for fish bones, 300 or 600 kg N ha^{-1} as compared with 262 or 524 kg N ha^{-1}, fish bones gave higher yields for most of the harvests (Figure 3). Across N-levels and drying status of materials, the average total above-ground DM yield where fish bones were applied was 13.10 g (3.3 g/pot), compared with 10.34 g for Calcinit (2.6 g per pot), and 10.25 g for algae fibre (2.6 g per pot). Even if considerably less N was applied with algae fibre than with Calcinit (190 or 380 vs. 300 or 600 kg N ha^{-1}), the average total above-ground DM yield was almost equal to the yield from Calcinit (Figure 3). These yield levels may be converted to yields per ha by dividing the weight in g by 4 (number of replicate pots) and multiply with a factor of 3 333 333.33. This factor is the weight of soil per ha if we assume that the nutrient uptake is taken

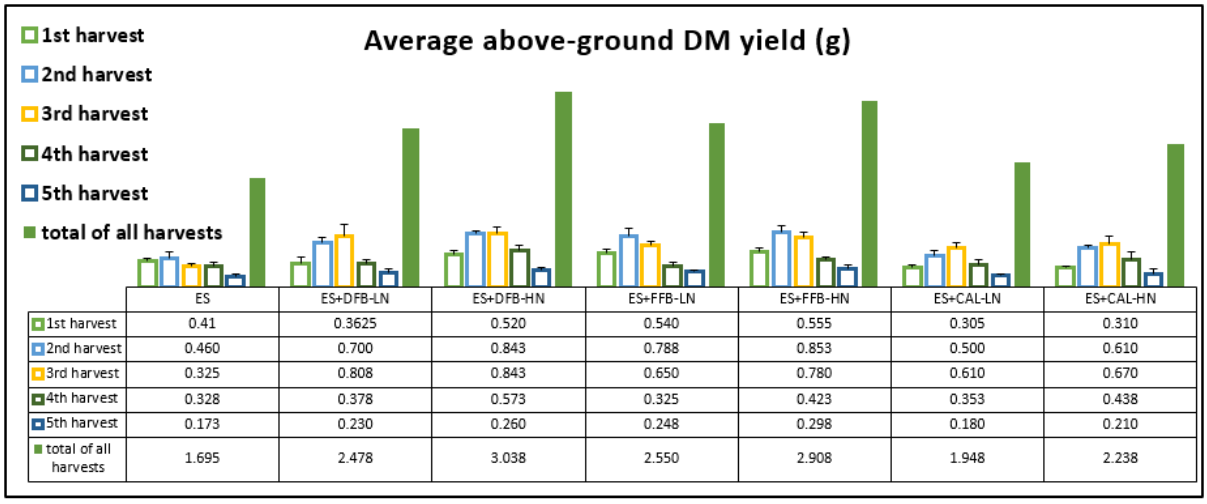
from the upper 20 cm and the soil bulk density = 1 kg/dm^3 soil = 2 000 000 kg soil per ha, divided by 0.6 which was the weight of soil in each pot. Using this factor, the total production of aboveground dry matter was 7 166 kg/ha without any fertiliser, 10 916 with fish bones, 8 616 with Calcinit and 8 541 kg/ha with algae fibre. The values obtained in a pot experiment are of course highly artificial and should not be directly compared with field results. Still, it is interesting to see that the value that was achieved for fish bones comes close to a value recorded with intensive growing conditions in practice; 11.1 tons of DM per ha with perennial ryegrass in southern Norway (Nærland, 2018).

The average yield increases across low and high N levels and drying status of materials, in % of the control yield with no fertiliser, was 52% for fish bones, 20% with Calcinit and 19% with algae fibre.

The stubble weight (in total across all treatments, 23.65 g) was about 20% of the total dry matter production (122.61 g). Since the stubble was present at each harvest, it may be more interesting to focus on how the yields of the leafy canopy developed over the experimental period. Hence, in further sections variations in yields between different harvests for each type of fertiliser are presented *without* the weight of the stubble, as an average value of aboveground plant material (DM) per pot (Figures 4 - 8).

3.3.3 Growth effect of fish bones

Fish bones (dried or fresh) had a very positive effect on DM yield, particularly at the first three harvests (Figures 4 and 5). Statistically significantly higher yields were achieved at the second and third harvests, with no significant variations between N levels or drying of the material (Figure 5). At the fourth harvest, there was a significant yield increase with high N level applied with dried fish bones. This possibly indicates that the N concentration in fresh fish bones may have been somewhat underestimated by the chemical analysis, which was conducted with material dried at 30 and 105 °C. If our assumption was correct, that drying of fish bones would decrease the N concentration as shown for fish bones conserved with formic acid (section 3.1), the growth effect of fresh fish bones should have been higher than the effect of dried fish bones. However, this seems not to be the case. Since no material is left of the fish bones which were used in this study, we are not able to explain this result in more detail.



	ES	ES+DFB-LN	ES+DFB-HN	ES+FFB-LN	ES+FFB-HN	ES+CAL-LN	ES+CAL-HN
1st harvest	0.41	0.3625	0.520	0.540	0.555	0.305	0.310
2nd harvest	0.460	0.700	0.843	0.788	0.853	0.500	0.610
3rd harvest	0.325	0.808	0.843	0.650	0.780	0.610	0.670
4th harvest	0.328	0.378	0.573	0.325	0.423	0.353	0.438
5th harvest	0.173	0.230	0.260	0.248	0.298	0.180	0.210
total of all harvests	1.695	2.478	3.038	2.550	2.908	1.948	2.238

Figure 4. Above-ground yields of dry matter (DM, g/pot), stubble excluded, for ryegrass amended with no fertiliser (ES) or low or high levels of N applied as dried or fresh fish bones (262 or 524 kg N ha^{-1}) or Calcinit (300 or 600 kg N ha^{-1}). Error bars represent the means ± SD (n = 4).

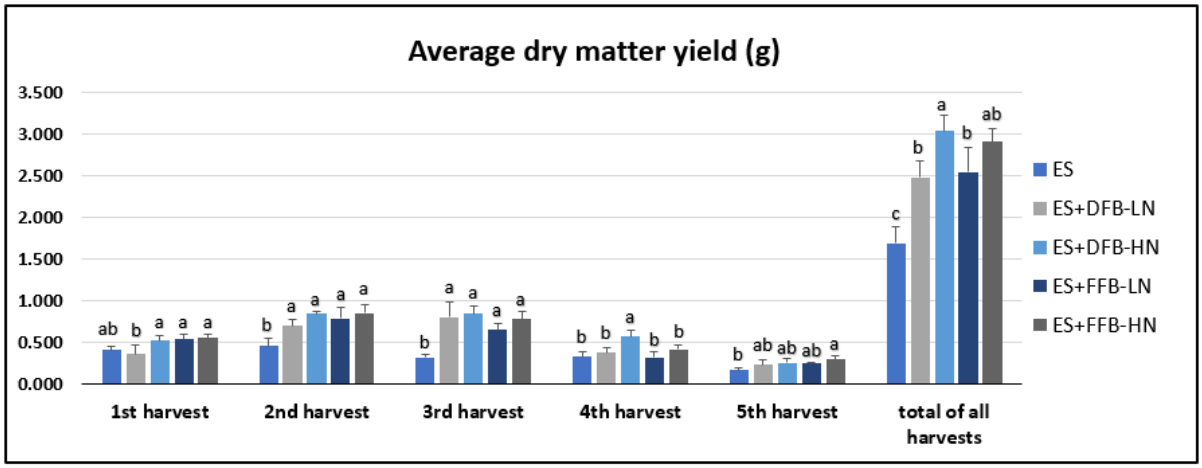


Figure 5. Above-ground yields of dry matter (DM, g/pot), stubble excluded, for ryegrass amended with no fertiliser (ES) or low or high levels of N (262 or 524 kg ha^{-1}) applied as dried or fresh fish bones (FB). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences between treatments within each harvest date, based on Tukey t-test (P = 0.05).

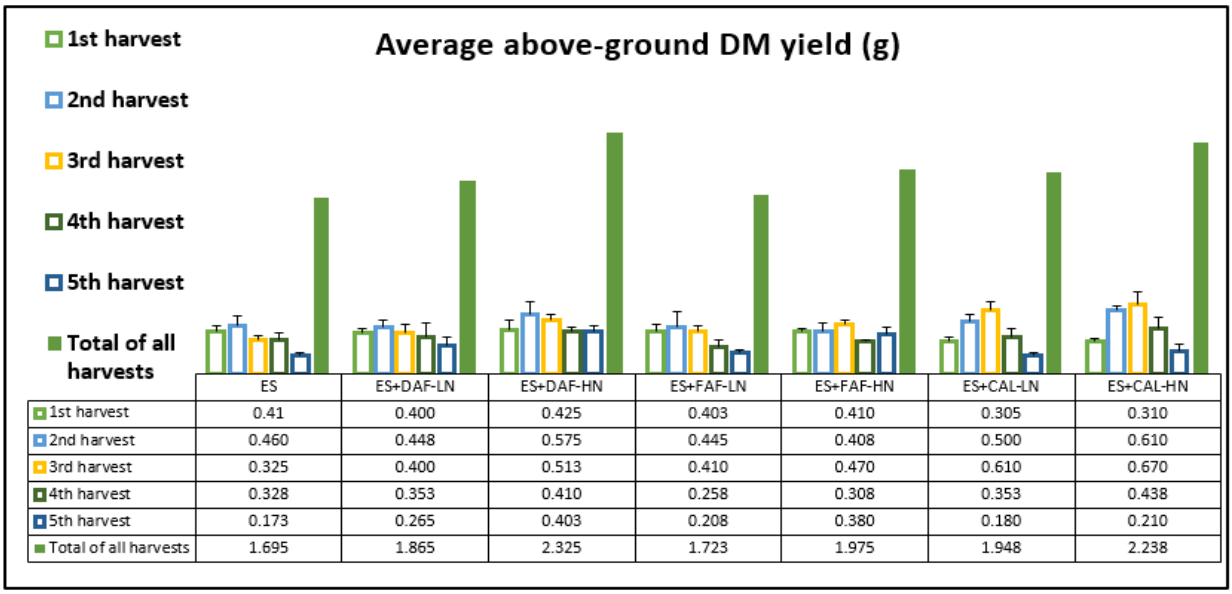
In general, fish bones as fertiliser provided clearly better yields than Calcinit; on average 2.5 - 3 g DM of leaves and stems per pot (Figure 5), as compared to about 2.1 g of DM per pot for Calcinit (Figure 8). This is likely due to the application of P with the fish bones (Table 4). The soil concentration of P expected to be available for plant uptake (P-AL) was medium to low; which may explain the good growth performance of ryegrass with this fertiliser.

In the control treatment, the yield was highest at the second harvest, then declined by about 1/3 on third harvest and maintained there at the fourth harvest; then declined by about 1/2 at the final harvest (Figure 4). This clearly shows that the soil became very depleted in nutrients during the experimental period. With fish bones, the yields were also most often highest at the second harvest and maintained high also at the third harvest. Then they levelled off quite rapidly, in a pattern quite similar to the control treatment. The yield at the final harvest was clearly lower than at the fourth harvest. Calcinit had a less rapid growth effect than fish bones. The yields with Calcinit were also higher at the second than at the first harvest but clearly higher at the third harvest than at the second. The reduction in yield from third to the fourth harvest was somewhat less than for control and fish bones, while the low yield at the final harvest shows that also in this treatment, the soil + fertiliser nutrients were clearly depleted at the end of the study.

The total yields of leaves and stems (sum of all harvests excluding stubble) with high N of fish bones was about 1.8-times higher, about 3.0 g DM per pot, as that which was produced with no fertilisation, which was about 1.7 g of DM per pot (Figure 4).

3.3.4 Growth effect of algae fibre

The growth effect of algae fibre was much less than for fish bones, but comparable with Calcinit, in spite of that the amounts of N applied were quite much lower: 190 or 380 kg N ha^{-1} as compared with 300 and 600 kg. Yields increased with increased amounts of algae fibre applied (Figure 6). Statistical analysis showed that algae fibre with high N level increased the yield levels significantly as compared to the control treatment at third harvest, and dried algae fibre with high N level also at the final harvest (Figure 7).



	ES	ES+DAF-LN	ES+DAF-HN	ES+FAF-LN	ES+FAF-HN	ES+CAL-LN	ES+CAL-HN
1st harvest	0.41	0.400	0.425	0.403	0.410	0.305	0.310
2nd harvest	0.460	0.448	0.575	0.445	0.408	0.500	0.610
3rd harvest	0.325	0.400	0.513	0.410	0.470	0.610	0.670
4th harvest	0.328	0.353	0.410	0.258	0.308	0.353	0.438
5th harvest	0.173	0.265	0.403	0.208	0.380	0.180	0.210
Total of all harvests	1.695	1.865	2.325	1.723	1.975	1.948	2.238

Figure 6. Above-ground yields of dry matter (DM, g/pot), stubble excluded, for ryegrass amended with no fertiliser (ES) or low or high levels of N applied as dried or fresh algae fibre (190 or 380 kg N ha^{-1}) or Calcinit (300 or 600 kg N ha^{-1}). Error bars represent the means ± SD (n = 4).

Also, for algae fibre, the yield of leaves and stems was most often highest at the second harvest, but the increase from the first harvest was not very high. What made the production of dry matter higher across five harvests with algae fibre higher than for the control, was a long-term effect where the yields maintained a quite stable level even at the final harvest, whereas in the control the yields levelled off (Figure 6). With high amounts of algae fibre applied, the yields were nearly equal or higher at the final than at the fourth harvest. At the final harvest, the yield with algae fibre was clearly higher than with Calcinit. This is a different pattern of growth effect as compared with the other treatments, and shows that the algae fibre has a rather slow, but long-term growth effect.

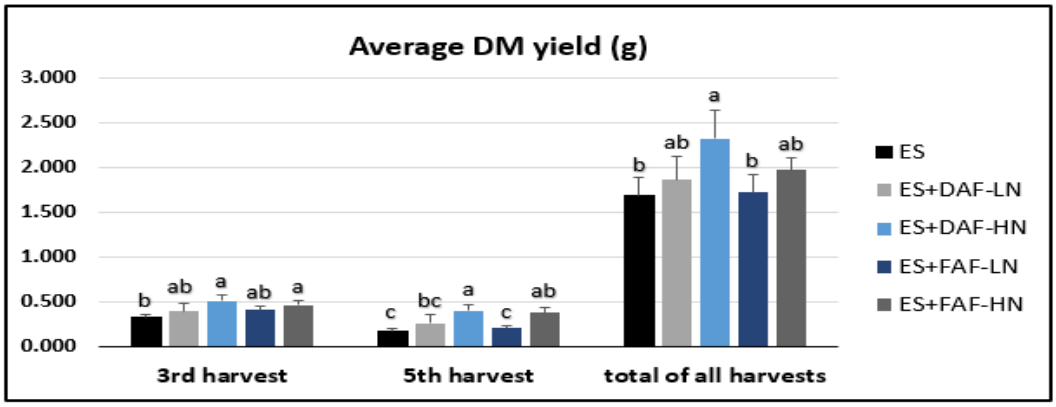


Figure 7. Above-ground yields of dry matter (DM, g/pot), stubble excluded, for ryegrass amended with no fertiliser (ES) or low or high levels of N applied as dried or fresh algae fibre (AF) (190 or 380 kg N ha^{-1}). Only harvests where statistically significant differences between treatments were found are shown. Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences between treatments within each harvest date, based on Tukey t-test (P = 0.05).

3.3.5 Growth effect of calcium nitrate

Calcinit, applied in low or high amounts corresponding to 300 or 600 kg N ha^{-1} decreased the yield of ryegrass at the first harvest as compared with the control (Figure 8). The difference was statistically significant and somewhat surprising. It may indicate that this mineral fertiliser may have some temporary negative effect on plant growth. At later harvests, the yields were as expected, clearly higher with Calcinit (Figure 6), and statistically significant differences were obtained at the third harvest (Figure 8). For Calcinit, we could also see a clear positive effect of increasing the level of N for the sum of all harvests (Figure 8).

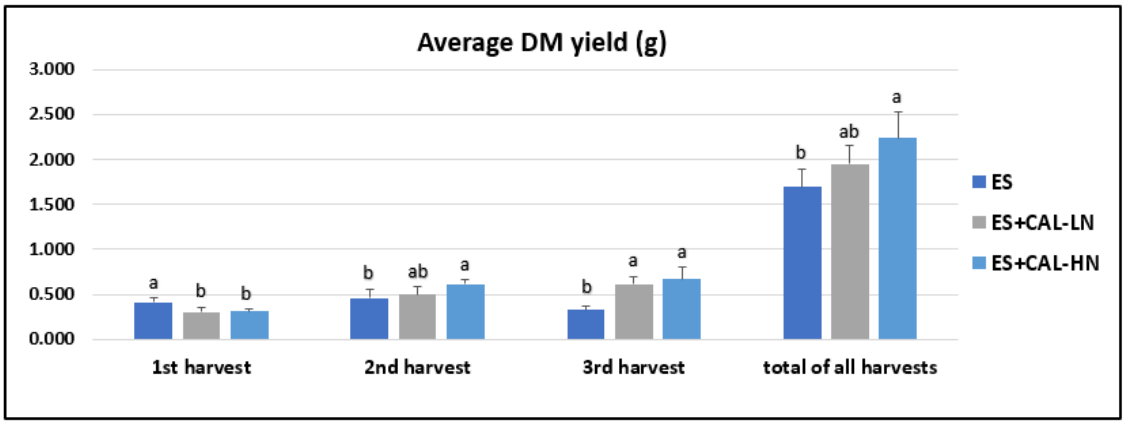


Figure 8. Above-ground yields of dry matter (DM, g/pot), stubble excluded, for ryegrass amended with no fertiliser (ES) or low or high levels of N applied as Calcinit (300 kg N or 600 kg N ha^{-1}). Only harvests where statistically significant differences between treatments were found are shown. Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences between treatments within each harvest date, based on Tukey t-test (P = 0.05).

3.3.6 Nitrogen utilisation with different fertilisers

The apparent N recovery (ANR), which shows how much of the applied N was utilised to produce above-ground plant tissue, was about 4-fold higher for fish bones than for Calcinit (Figure 9). As expected, the N was better utilised with low N application. More than 60% of the applied N with fish bones was then recovered in above-ground plant material. With high N level, this value decreased to about 54% for fish bones. The fish bones had a C:N ratio of 3 (Table 4) (section 3.1), which is less than the optimal value of 24:1 commonly referred to for soil organisms (Weil and Brady, 2016). With organic materials of low C:N, enough N is present to meet the needs of soil microorganisms, while also concurrently feeding crop plants. With the application of fish bones, lots of nitrogen became available in the soil for the growth of ryegrass plants, as was observed here by enhanced N uptake in plants fertilised by fish bones (discussed later in section 3.6.4).

The ANR with algae fibre showed a *negative* trend with a maximum negative value (-19%) for fresh algae fibre with low application of N (Figure 9). A positive value (12.5%) was observed only with dried algae fibre with high nitrogen application. The C:N ratio of algae fibre was about 22 (Table 4). This is not so far from the ideal value of 24, but the N in this material may not have been easily available for plants. The negative or only weakly positive ANR values, and generally slow, but clearly positive growth effect of algae fibre in ryegrass, may indicate that microorganisms competed with the plants for available N during the start of the experiment, while the organic matter was decomposed to

release N from the algae fibre. Even though some of the soil N and algae fibre-derived N was likely consumed by microorganisms, still some was available, which led to better growth of ryegrass plants.

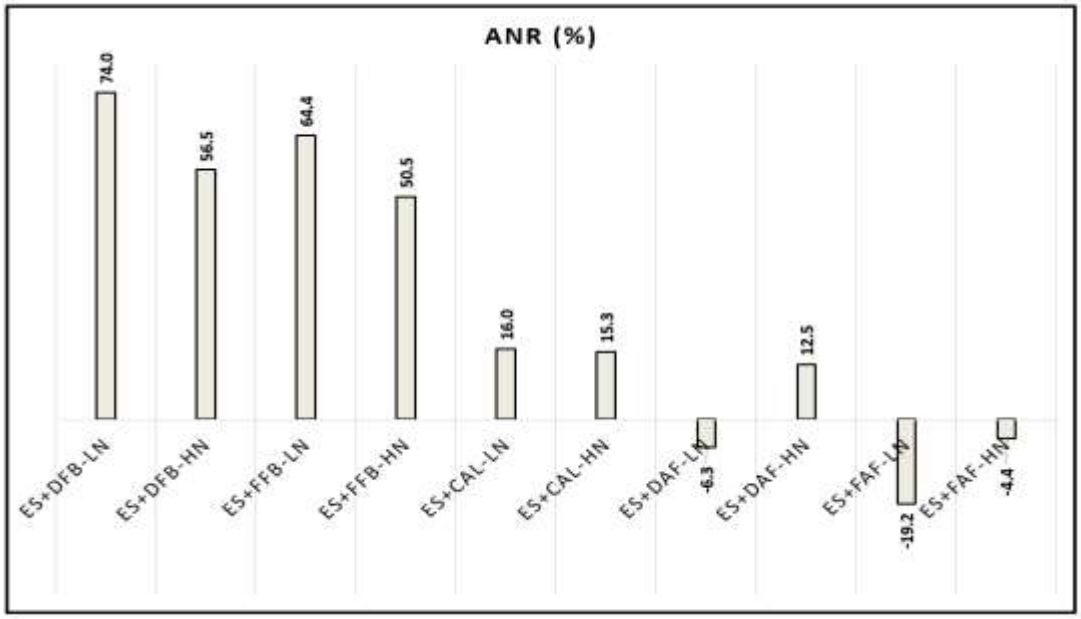


Figure 9. Apparent nitrogen recovery (ANR, %) in ryegrass plants amended with low and high N levels (LN or HN) of dried or fresh fish bones (DFB or FFB), Calcinit (CAL) or dried or fresh algae fibre (DAF or FAF). ANR = N uptake in a fertilised treatment minus N uptake in the control, divided by the amount of N applied with fertiliser times 100.

For Calcinit, the utilisation was surprisingly low with both N levels; only about 15.5% of the applied N was recovered in the above-ground plant material (Figure 9). This may show that when other nutrients, such as P and K, were not applied, the plants were not able to utilise the applied N very well.

3.3.7 Relationships between N application and above-ground plant material production

Linear regressions fitted to the N fertilisation and mean above-ground DM yield per pot, excluding the stubble (as presented in Figures 4 - 8) did not give significant relationships at the first or second harvest (r^2 = 2.4 and 29.3, Figure 10 A and B). At the third and fourth harvests, significant relationships were found (r^2 = 47.9* and 47.4*, $P < 0.05$, Fig 10 C and D). For the average accumulated harvest, the relationship was also significant, and the N level explained about 45% of the variation (r^2 = 46.4*, $P < 0.05$, Figure 10 F).

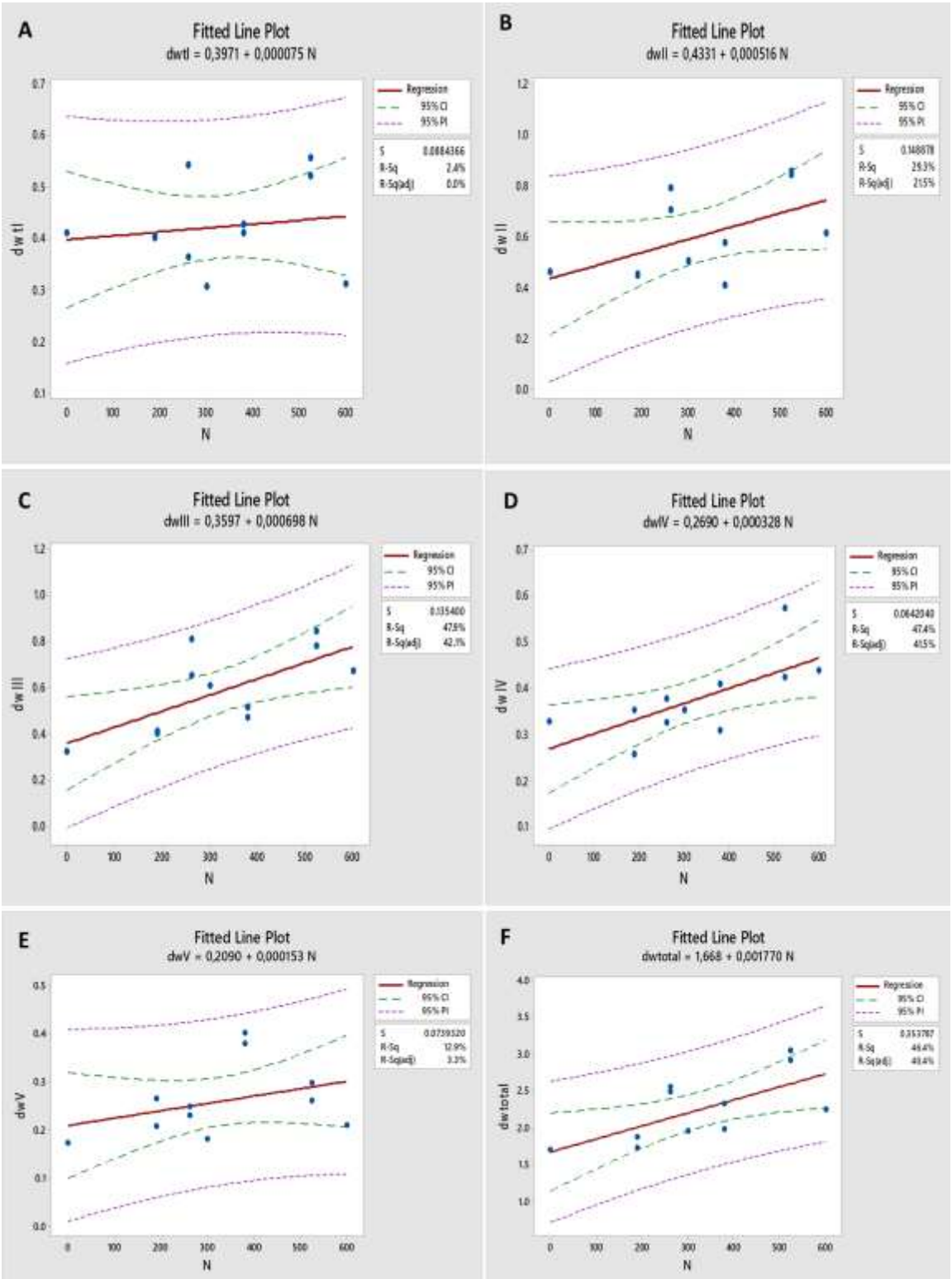


Figure 10. Fitted line plots showing the relationships with linear regression equations between levels of nitrogen application (kg N/ha) and the mean above-ground DM yield (g per pot) ***<u>of leaves and stems</u>*** *at each harvest and for a total of all harvests.* ***(A)*** *DM yield at first harvest,* ***(B)*** *DM yield at second harvest,* ***(C)*** *DM yield at third harvest,* ***(D)*** *DM yield at fourth harvest,* ***(E)*** *DM yield at fifth harvest,* ***(F)*** *DM yield for total of all harvests.*

When the stubble was included in the total DM yield (as presented in Figure 3), a significant relationship was again found between the N fertilisation and the total DM yield (sum of 4 replicate pots per treatment) (r^2 = 47.8*) (Figure 11 C). However, for the stubble weight alone, no significant relationship was observed between N fertilisation and yield (r^2 = 8.0, Figure 11 B). For comparison, also the relationship between the amount of N applied and the accumulated aboveground yield excluding the stubble is shown in Figure 11 A.

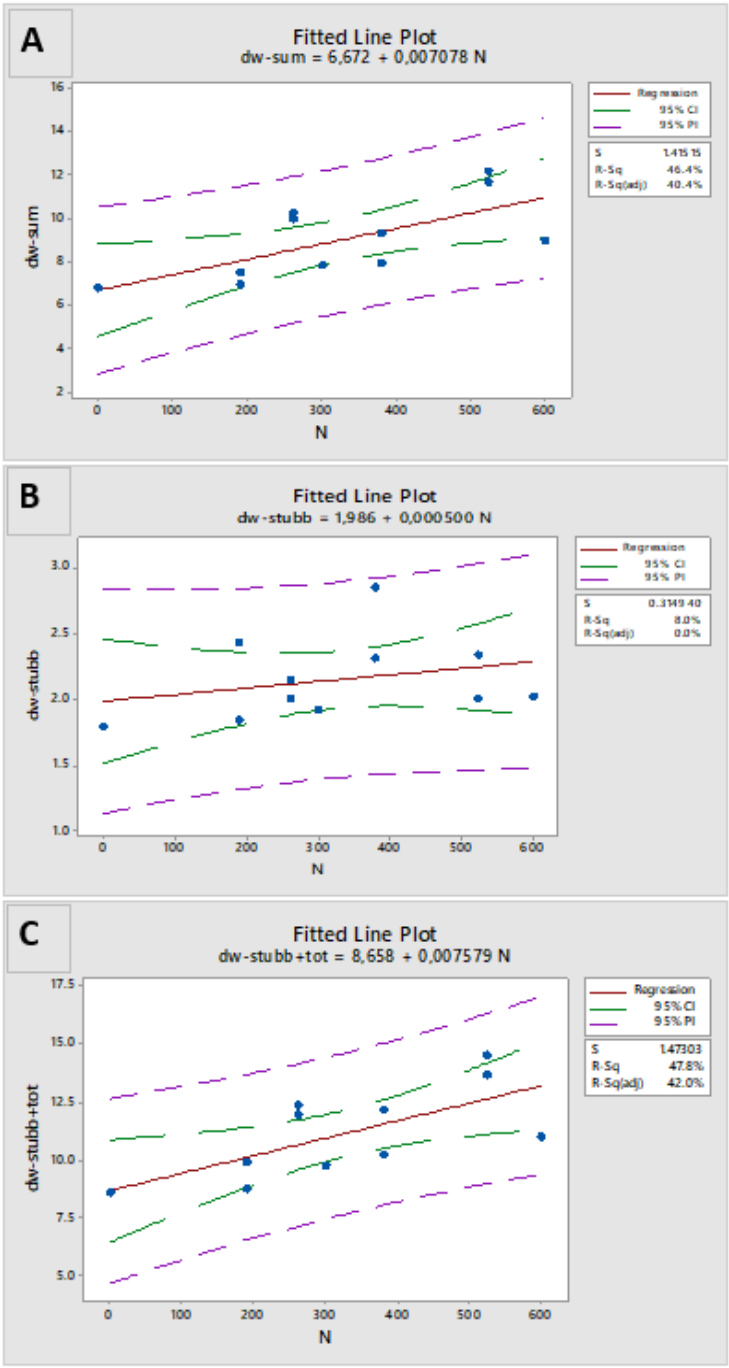
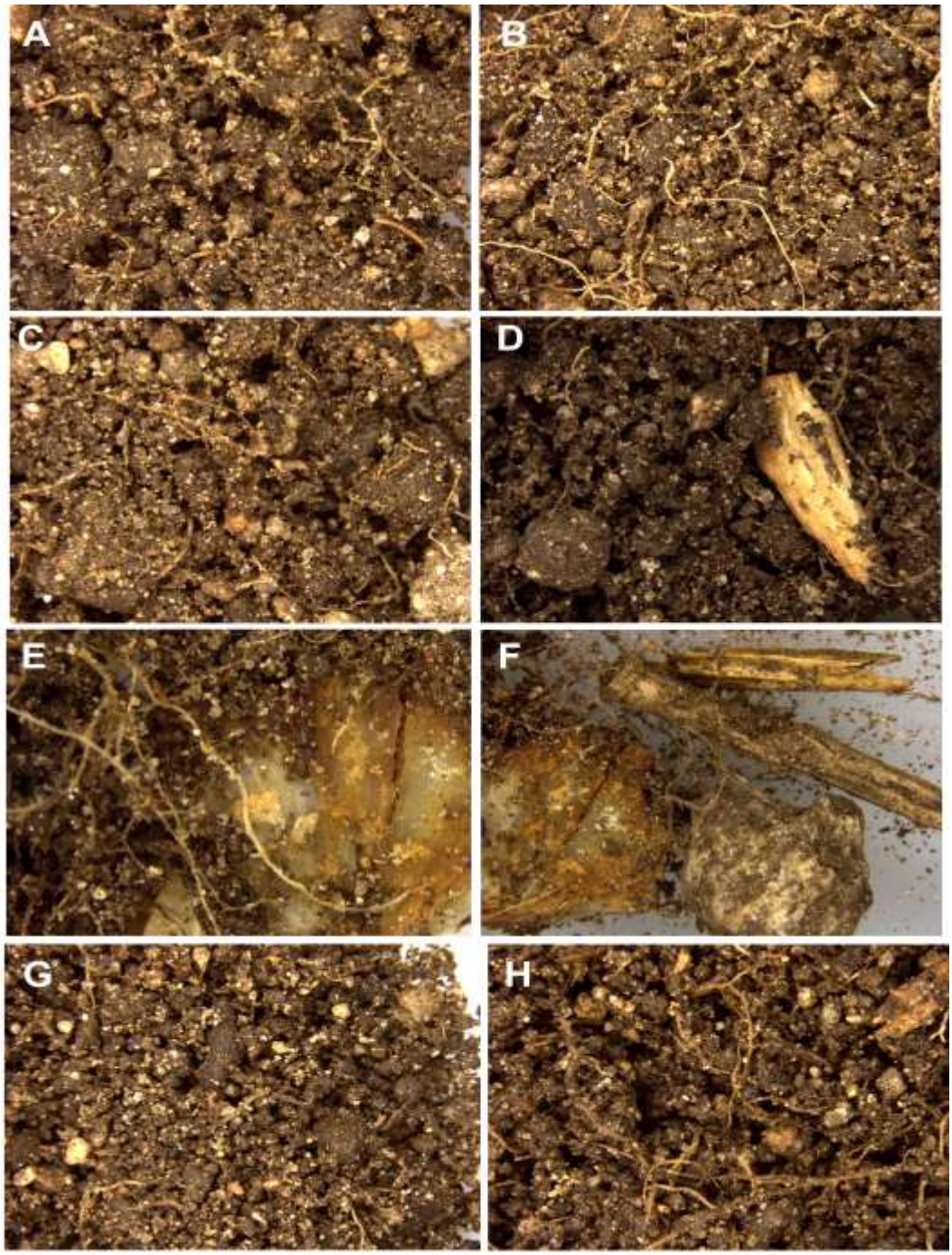


Figure 11. Fitted line plots showing the relationships with linear regression equations between levels of nitrogen application (kg N/ha) and the above-ground DM yield across all harvests for leaves and stems, the yield including the stubble at the final harvest, and the yield of stubble at the final harvest. ***(A)*** *Sum of leaves and stems at all harvests* ***(B)*** *stubble only, at the final harvest,* ***(C)*** *total aboveground weight at final harvest including stubble.*

3.4 Microscopic examination of soil

The examination of soil in the microscope revealed some differences between unfertilised soil and amended soils (Picture 6). Remains of fish bones were observed in soil amended with high amounts of fish bones (Picture 6 C, D, E and F). Soil amended with a high amount of algae fibre (Picture 6 G and H) appeared relatively dark in colour compared to the experimental soil with no fertiliser (Picture 6 A) and Calcinit (Picture 6 B).



Picture 6. The appearance of soil samples fertilised with high levels of fresh fish bones (C, E and F), dried fish bones (D), dried or fresh algae fibre (G and H), and Calcinit (B) after the final harvest of ryegrass, as compared with unfertilised soil (A). Images are taken by Anne de Boer, NIBIO, Tingvoll.

3.5 Effects of fertilisers on soil characteristics

3.5.1 Soil pH

At the start of the experiment, soil pH (on average for 4 parallel samples) was 5.3. After about 4 months of plant growth, soil pH ranged from 5.1 to 6.8 (Figure 12 A). In the control soil, the pH value was not affected by the growth of ryegrass. The lowest values were observed for soil amended with Calcinit. Soil pH was not affected by the high content of calcium in fish bones (Table 4), and the pH in this soil was equal to the control.

For algae fibre, which had a pH of about 10 (Table 4), the soil pH increased significantly (Figure 12 B), and even more with the higher application. Soil pH with the low application was 6.2, and with high N level it was 6.8. Soil pH above 7.5 may affect negatively the uptake of P and micronutrients such as Fe, B, Zn and Cu, so this effect of algae fibre should be considered while using algae fibre in high amounts as fertiliser. Concurrently, this shows that algae fibre may be applied as a liming agent to soils where a higher pH would be beneficial.

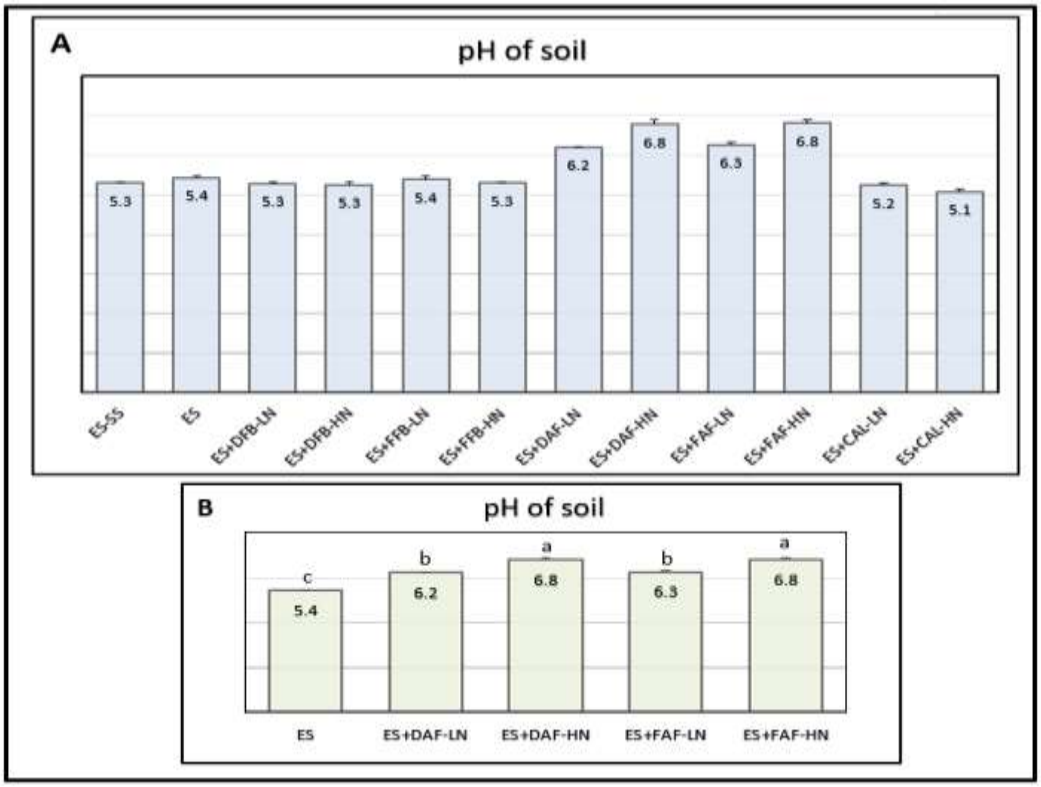


Figure 12. Soil pH before and after growth of ryegrass in pots for about 4 months.
***(A)** Experimental soil at the start of the study (ES-SS), experimental soil at the end of the study (ES), soil amended with dried or fresh fish bones (DFB or FFB), soil amended with dried or fresh algae fibre (DAF or FAF), and soil amended with Calcinit (CAL), with low or high application of each fertiliser (LN or HN).*
***(B)** Statistical evaluation of pH in the soil at the end of the study (ES), and soil amended with dried or fresh algae fibre (DAF or FAF). Error bars represent the means ± SD (n = 4; except ES+CAL-LN where n = 3). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).*

3.5.2 Soil organic matter

The initial average value of soil organic matter (SOM) as measured by loss-on-ignition was 11.5% (Figure 13) with a range between 11.0 and 12.1. Regardless of no fertilisation and no application of organic matter, this value increased was higher in the unfertilised at the end of the study. The average value was to 12.1, with a range between 11.4 and 12.8. This is likely due to that the ryegrass produced soil organic matter during growth. With 444 g of dry soil per pot initially, the amount of soil organic matter produced comprises about 2.6 g per pot. Each pot contained 444 g dry soil with on average 11.5 % SOM = 51.1 g SOM at the start of the experiment.

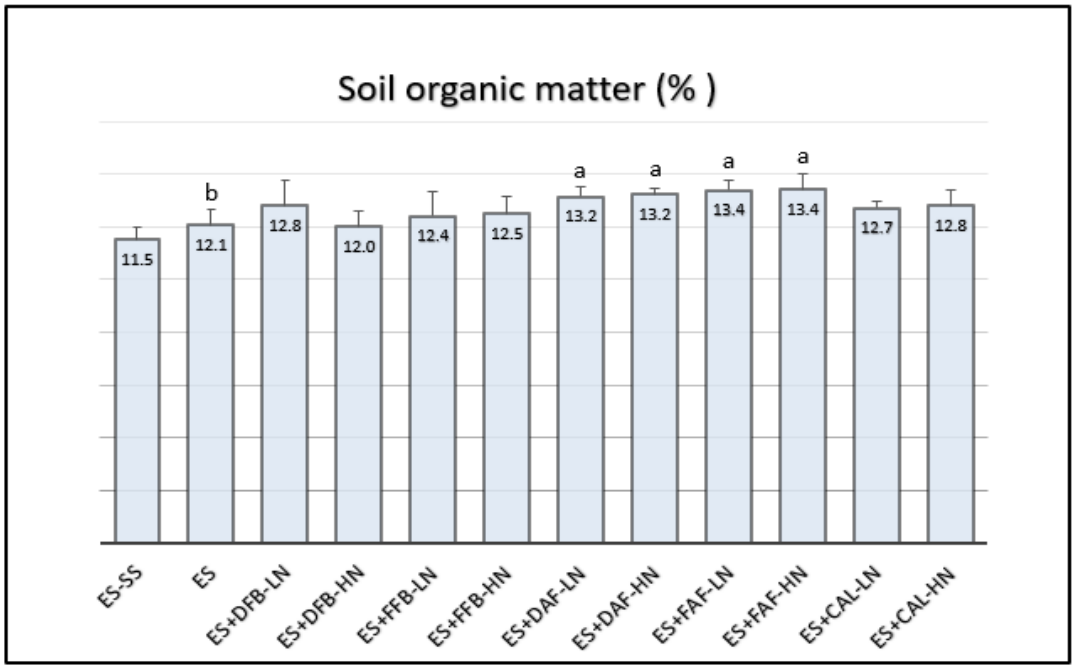


Figure 13. The concentration of soil organic matter (SOM) before and after the growth of ryegrass in pots for about 4 months. Experimental soil at the start of the study (ES-SS), experimental soil at the end of the study (ES), soil amended with dried or fresh fish bones (DFB or FFB), soil amended with dried or fresh algae fibre (DAF or FAF), and soil amended with Calcinit (CAL), with low or high application of N (LN or HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences between experimental soil and soil amended with algae fibre, based on Tukey t-test (P = 0.05).

With Calcinit, the average value of SOM was 12.7% with low N, and 12.8% with high N (Figure 13). This increase is likely due to SOM produced from the ryegrass plants, which was supported by Calcinit. The increase in SOM was about 5.5 g SOM per pot. With fish bones, SOM values varied between 12.0 and 12.8. Some organic matter was applied with the fish bones; 34 g/100 g of DM (Table 4), and the plants grew vigorously. Hence, we might have expected a somewhat stronger increase in SOM with fish bones than was obtained with Calcinit (Figure 13). However, the root: shoot relationship may be been affected by the fertilisation, with possibly fewer roots in the relatively better-fertilised ryegrass plants amended with fish bones.

A significant increase in SOM was obtained with application of algae fibre (Figure 13). However, the values did not vary between lower and higher N applications. With low N level, the SOM was 13.2% and 13.4% for dried and fresh material, and with high N level, the values were again 13.2% for dried and 13.4% for fresh material. The high values of SOM in algae fibre amended soils can be due to the high amount of organic matter in algae fibre, which was about 55 g/100 g DM (Table 4) (section 3.1).

3.5.3 AL-extractable macronutrients and Na

3.5.3.1 P-AL concentrations

With the application of fish bones, soil P-AL concentrations increased significantly, from an initial average value of 3.6 mg/100 g soil to values between 8.2 and 27.5 (Table 7, Figure 14 A). Higher applications of fish bones provided higher values. The drying at 105 °C seems to have had a positive effect on this fertilisers' capacity to increase soil P-AL. With no fertiliser, the P-AL decreased slightly, from 3.6 to 3.1 (Table 7), and with Calcinit, the values decreased somewhat more to 2.9 (Figure 15A). With algae fibre, the P-AL values were maintained with low N application and increased slightly with high applications. The P-AL concentrations were significantly higher in algae fibre amended soil than in the control soil (Figure 16 A).

Table 7. AL-extractable macronutrients and sodium, mg/ 100g air-dried soil, before and after growth of ryegrass in pots for about four months.

Sample Code	P-AL	K-AL	Ca-AL	Mg-AL	Na-AL
ES-SS	3.6	5.1	140.0	6.6	6.3
ES	3.1	3.2	140.0	5.5	7.1
ES+DFB-LN	11.1	2.8	152.5	5.3	6.7
ES+DFB-HN	27.5	3.0	190.0	5.8	8.0
ES+FFB-LN	8.2	2.9	150.0	5.6	6.5
ES+FFB-HN	13.2	3.1	160.0	5.8	6.2
ES+DAF-LN	3.4	39.8	182.5	22.3	21.3
ES+DAF-HN	3.9	90.8	212.5	36.5	33.8
ES+FAF-LN	3.2	44.5	177.5	20.8	21.8
ES+FAF-HN	3.6	107.5	210.0	35.3	38.0
ES+CAL-LN	2.9	3.4	153.3	5.2	6.7
ES+CAL-HN	2.9	2.7	165.0	5.1	6.1

Average values (n = 4; except ES+CAL-LN where n = 3). AL ammonium-acetate lactate; Ca Calcium, Na sodium, P phosphorous, K potassium, Mg magnesium, experimental soil at the start of the study (ES-SS), experimental soil at the end of the study (ES), soil amended with dried or fresh fish bones (DFB or FFB), soil amended with dried or fresh algae fibre (DAF or FAF), soil amended with Calcinit (CAL), with low or high application of N (LN, HN).

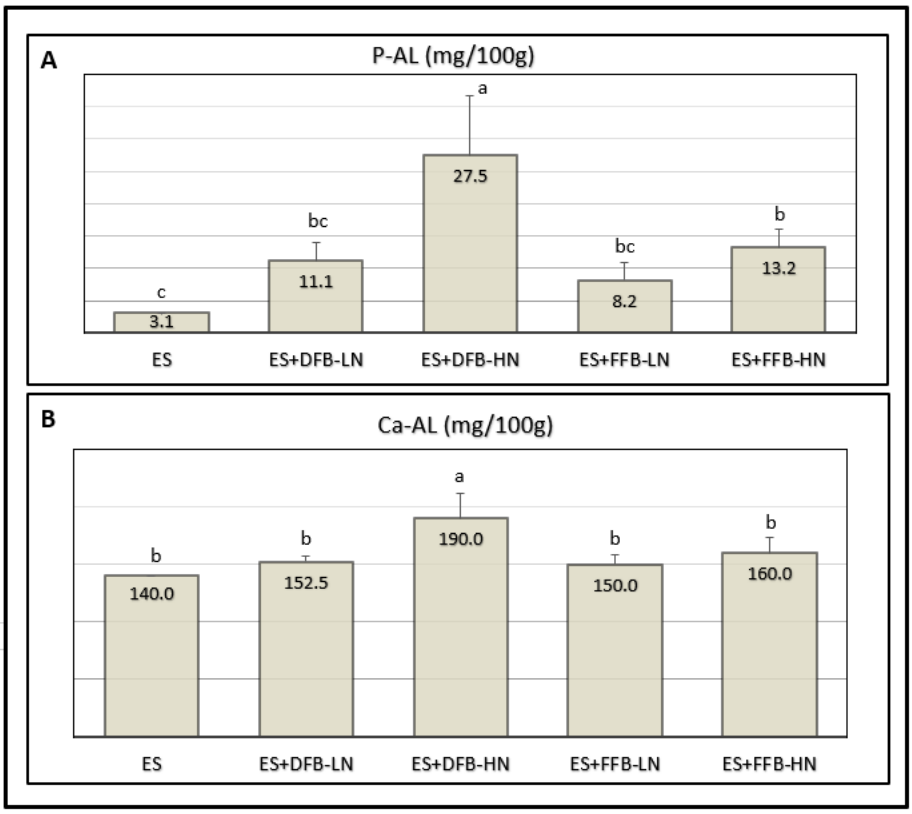


Figure 14. AL-extractable phosphorus (P) and calcium (Ca) in soil amended with fish bones compared with unfertilised soil after growth of ryegrass for about four months. Experimental soil (ES), soil amended with dried or fresh fish bones (DFB or FFB), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).

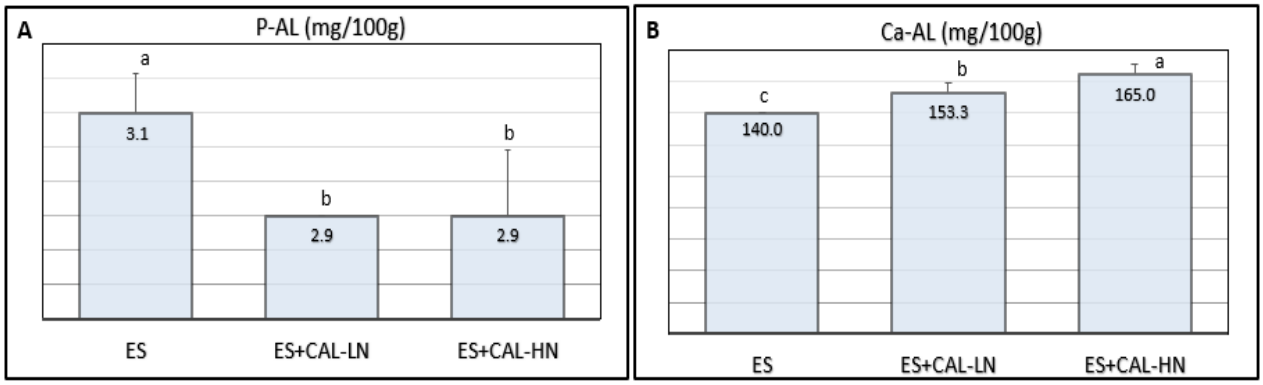


Figure 15. AL-extractable phosphorus (P) and calcium (Ca) in soil amended with Calcinit compared with unfertilised soil after growth of ryegrass for about four months. Experimental soil (ES), soil amended with Calcinit (CAL), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4; except ES+CAL-LN where n = 3). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).

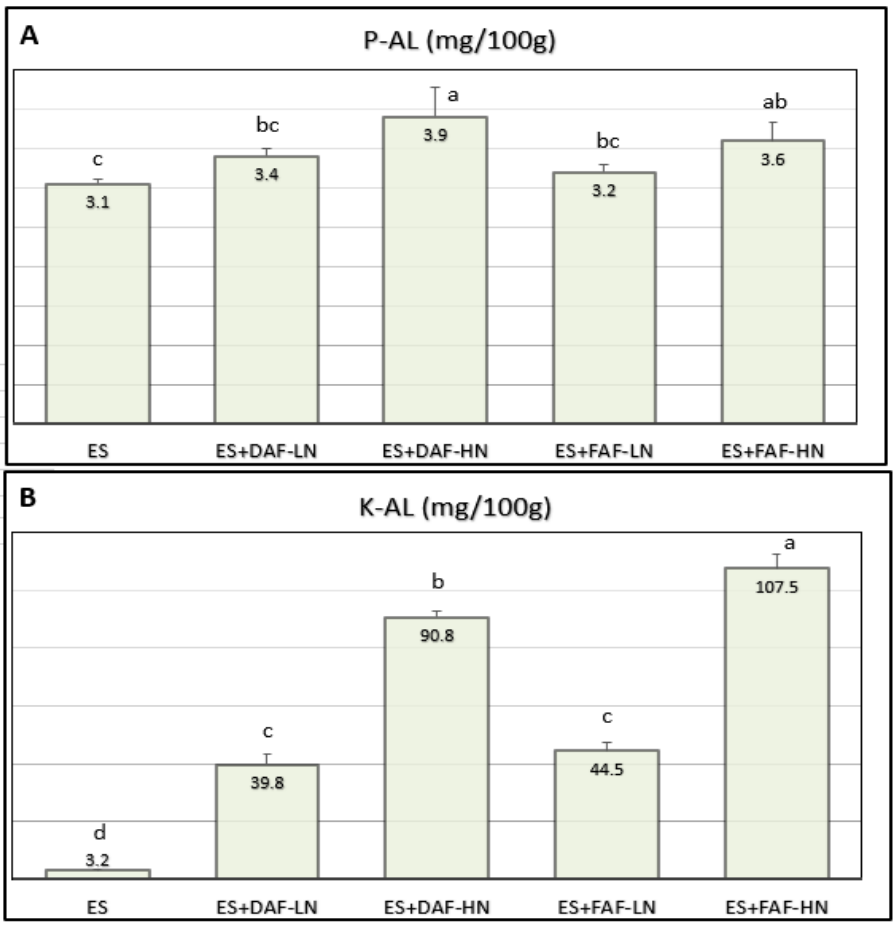


Figure 16. AL-extractable phosphorus (P) and potassium (K) in soil amended with algae fibre compared with unfertilised soil after growth of ryegrass for about four months. Experimental soil (ES), soil amended with dried or fresh algae fibre (DAF or FAF), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).

3.5.3.2 K-AL concentrations

With algae fibre, soil K-AL concentrations increased significantly, from an initial value of 5.1 mg/100 g air-dried soil to values about 40 to 45 for low application, and 90 - 108 for high application (Table 7, Figure 16 B). This was also confirmed by a former study, where K levels were significantly higher in seaweed-treated potato plots compared to control and conventionally fertilised plots (López-Mosquera and Pazos, 1997). In unfertilised soil, K-AL levels decreased from 5.1 to 3.2. A similar value was found after application of Calcinit, 3.4 mg K-AL/100 g soil with low, and 2.7 with high N application (Table 7). For fish bones, the values of K-AL also decreased significantly and varied between 2.8 and 3.1 (Table 7). This illustrates how ryegrass is efficient to harvest soil K, especially when significant amounts of N and P are applied.

3.5.3.3 Ca-AL concentrations

The initial concentration of Ca-AL in the experimental soil was very high; 140 mg/100 g soil (Table 7), and the value was similar in the control soil after the growth of ryegrass (Table 7). The concentration of total Ca was much higher in fish bones (300 g per kg DM) than in algae fibre (68 g per kg DM, Table 4). With algae fibre, 544 mg Ca per pot was applied with high N levels, and with fish bones, 900 mg per pot. The soil Ca-AL concentration increased significantly with high application of dried fish bones (Figure 14 B). For algae fibre, the concentrations increased more, to about 183 with low amounts applied, and about 213 with high amounts applied (Figure 17 B). The Ca-AL concentrations increased also with the application of Calcinit, to 153 and 165 mg/100 g air-dried soil with low and high N applications (Figure 15 B, Table 7). The calcium in fish bones seems to be somewhat less extractable in AL-solution than the calcium in Calcinit and algae fibre. The drying of fish bones did not decrease the AL-extractability of Ca (Figure 17 B).

3.5.3.4 Mg-AL concentrations

The initial concentration of Mg-AL in the experimental soil was 6.6 mg/100 g soil. In parallel to what was observed for K-AL, this value decreased to 5.5 without fertiliser application and even more to 5.2 and 5.1 with low and high applications of Calcinit (Table 7). The values decreased slightly also with the application of fish bones, ranging from 5.3 - 5.8. With the application of algae fibre, these values increased significantly, to about 21 with low, and 35 with high N application (Figure 17 A). This corresponds well with high levels of total Mg in algae fibre (Table 4); 200 mg of Mg was applied per pot with high applications of algae fibre.

3.5.3.5 Na-AL concentrations

Soil Na-AL concentrations should be kept low to avoid competing with the uptake of K. The initial value of Na-AL in experimental soil was 6.3. With no fertilisation, this value increased slightly to 7.1 (Table 7). With fish bones and Calcinit, the value was not notably affected, as concentrations varied between 6.1 and 8.0. With algae fibre, the values increased significantly, to about 21 with low and 35 with high N application (Figure 17 C). The concentrations of Na were not measured in the fertilisers used here, but obviously, they were high in algae fibre, and this characteristic should be observed in future studies to avoid imbalances in plant nutrition.

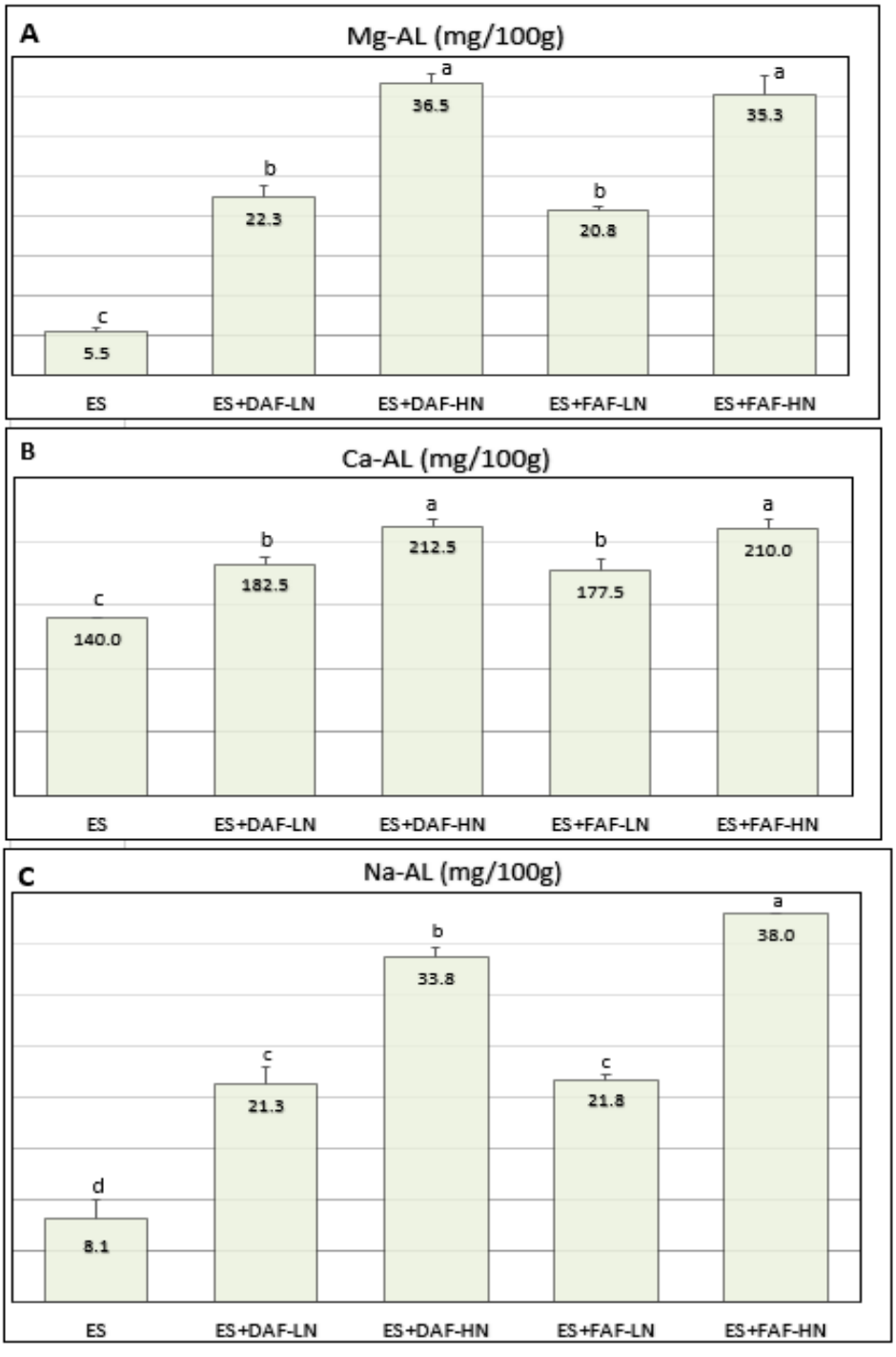


Figure 17. AL-extractable magnesium (Mg), calcium (Ca), and sodium (Na) in soil amended with algae fibre compared with unfertilised soil after growth of ryegrass for about four months. Experimental soil (ES), soil amended with dried and fresh algae fibre (DAF or FAF), with low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).

3.6 Effects of fertilisers on element concentrations in above-ground plant material

From each pot, the dry plant material (foliage + stubble) from all harvests was compiled and analysed for concentrations of elements (Tables 8 - 10, Figures 18 – 20).

3.6.1 Macronutrients and sodium

The total N concentration in the unfertilised (control) plants was 3.1% (of DM) (Table 8), which indicates that the control soil had a relatively high capacity to supply plants with N. With fish bones and Calcinit, the concentrations were slightly higher than control and varied between 3.2 and 3.5%. The algae fibre significantly reduced total N concentrations in above-ground plant material compared with the unfertilised plants (Figure 18 A).

Table 8. Concentrations of macronutrients and sodium (% of DM) in above-ground material of ryegrass.

Sample Code	N	P	K	Ca	Mg	S	Na
ES	3.1	0.265	2.18	0.985	0.327	0.290	0.488
ES+DFB-LN	3.5	0.345	1.74	1.007	0.336	0.237	0.599
ES+DFB-HN	3.4	0.500	1.37	1.191	0.388	0.232	0.519
ES+FFB-LN	3.2	0.407	1.75	1.040	0.346	0.266	0.556
ES+FFB-HN	3.5	0.470	1.38	1.060	0.376	0.223	0.616
ES+DAF-LN	2.5	0.321	4.86	0.463	0.262	0.321	0.158
ES+DAF-HN	2.6	0.359	5.15	0.341	0.256	0.366	0.243
ES+FAF-LN	2.5	0.376	5.05	0.421	0.237	0.315	0.166
ES+FAF-HN	2.4	0.427	5.31	0.304	0.240	0.325	0.173
ES+CAL-LN	3.3	0.218	1.88	1.216	0.333	0.243	0.484
ES+CAL-HN	3.4	0.198	1.47	1.272	0.310	0.207	0.438

Mean values (n = 4) of accumulated aboveground plant material, stubble included. Ca Calcium, N Nitrogen, Na Sodium, P Phosphorous, K Potassium, Mg Magnesium, S Sulphur. Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN).

The concentrations of P were higher in control, 0.27%, than with Calcinit, 0.22 and 0.20% with the low and high application (Table 8). With application of algae fibre, the values were significantly higher, ranging from 0.32% with low application of dried fibre to 0.43% with high-level fresh fibre (Figure 18 B). This may be due to that even if the P concentration in algae fibre was low as compared with the fish bones, more P than N was actually applied with this fertiliser (P:N ratio = 1.7). As expected, from the much higher P:N ratio of 17.5 in fish bones, P concentrations were significantly higher in ryegrass amended with fish bones. The values were 0.35 and 0.41% with low N level and about 0.5% with a high level of applied material (Figure 19 A).

The K concentrations were significantly affected by fertilisation. Without fertilisation, the K concentration was 2.2% of DM (Table 8). With the application of high amounts of P and N in fish bones, but no K, the K concentration was much lower (1.4 - 1.8%, Figure 19 B), and the same was valid for Calcinit (1.5 - 1.9%, Table 8). With application of algae fibre, the concentrations of K increased significantly and reached very high levels, ranging from 4.9 to 5.3% of DM (Figure 18 C).

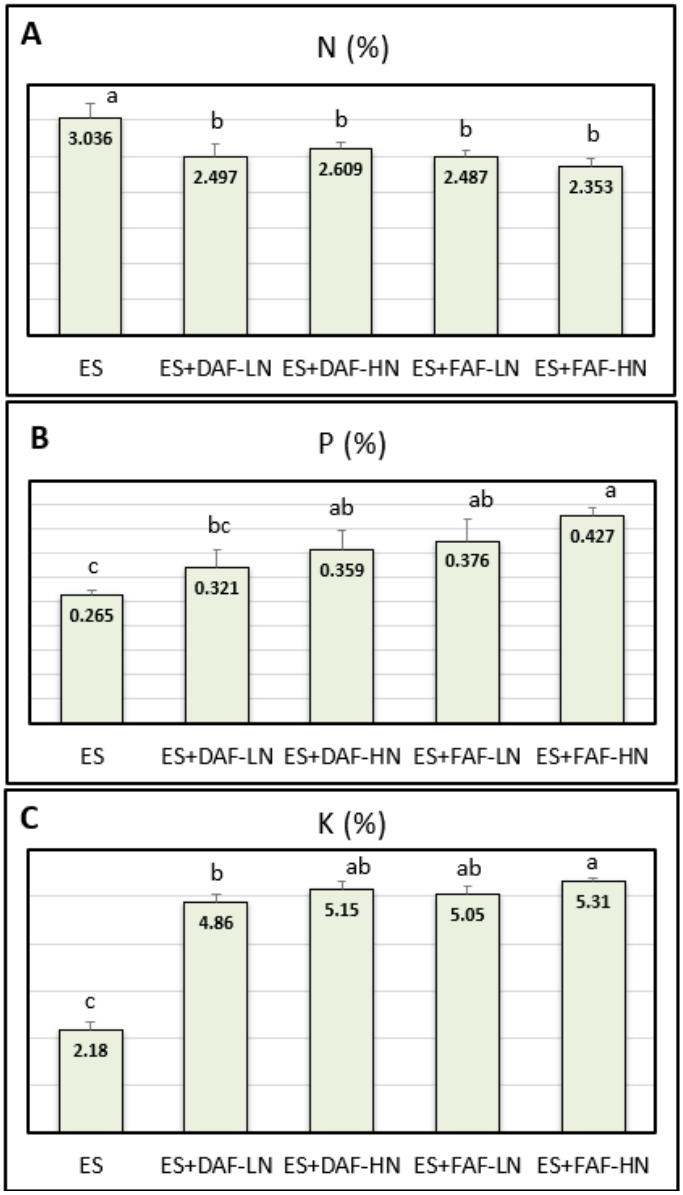


Figure 18. Concentrations of N, P and K (% of DM) in above-ground plant material of ryegrass (compiled from five harvests, stubble included) grown on unfertilised soil and soil amended with algae fibre (dried and fresh). Experimental soil (ES), soil amended with dried or fresh algae fibre (DAF and FAF), with low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences based on Tukey t-test (P = 0.05).

The concentration of Ca was 0.99% of DM in the unfertilised soil, which was quite similar with values obtained with application of fish bones and Calcinit. These values ranged from 1.01 to 1.19 with fish bones, and from 1.22 to 1.27 % with Calcinit for low and high applications (Table 8, Figure 19 D). With algae fibre, the average concentration across the treatments was only 0.38%, which was 2.6-fold lower than control, differing significantly (Figure 20 A). Since the algae fibre increased soil Ca-AL concentrations significantly (Figure 17B, section 3.5.3.3), this may seem to be a surprising result. However, it may be explained by the large uptake of K from algae fibre since monovalent cations (K^+) are taken up more easily than divalent cations (Ca^{2+}).

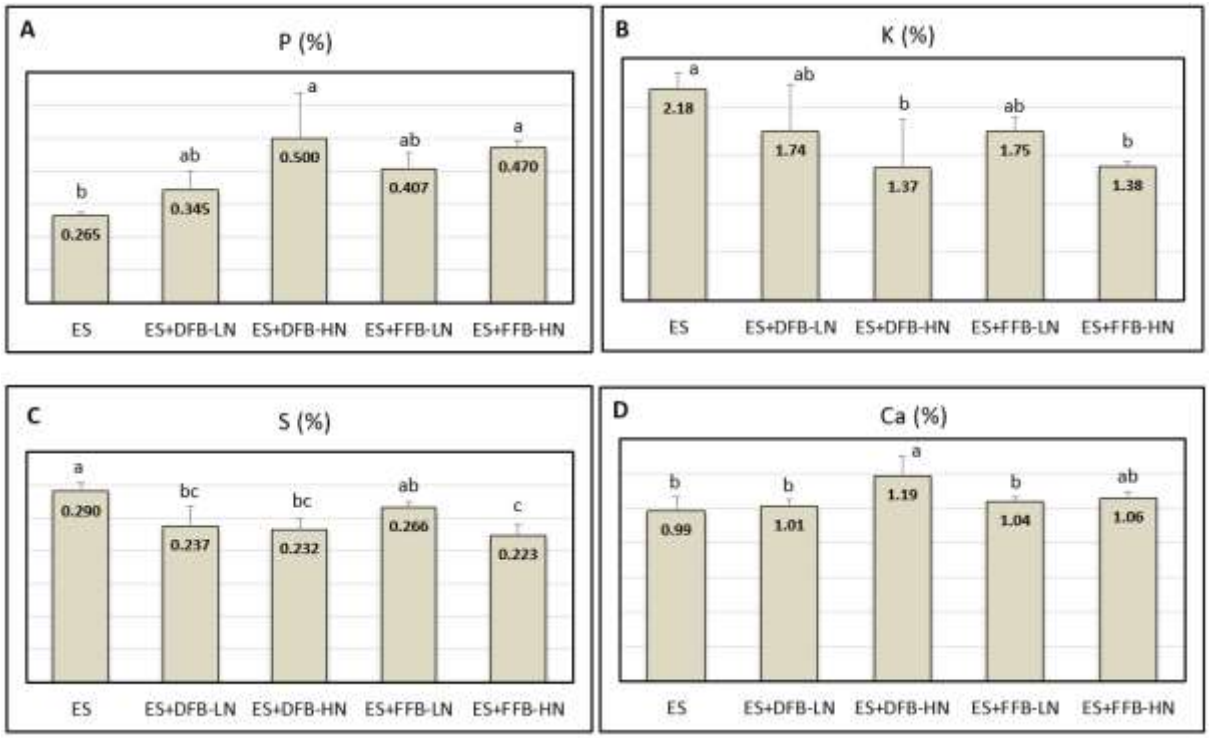


Figure 19. Concentrations of P, K, S and Ca (% of DM) in above-ground plant material of ryegrass grown on unfertilised soil and soil amended with fish bones (dried and fresh). Experimental soil (ES), and soil amended with dried or fresh fish bones (DFB or FFB), with low and high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences, based on Tukey t-test (P = 0.05).

The Mg concentration was about 0.33 % of DM and did not differ between unfertilised plants (control) and plants fertilised with Calcinit (Table 8). With fish bones, the Mg concentrations increased slightly, to 0.34% with low and 0.39% with high applications. With algae fibre, the values decreased significantly to 0.24 - 0.26% of DM (Figure 20B). This pattern is the same as was observed for calcium, where algae fibre increased soil Mg-AL concentrations significantly (Figure 17 A) but did not increase concentrations in the aboveground plant material (Figure 20B), which is likely due to high concentrations of K (Figure 18 C).

The S concentrations were somewhat lower in plants fertilised with fish bones and Calcinit (0.21 - 0.27 % of DM), as compared with the control (0.29%). (Table 8, Figure 19 C). With algae fibre, the values increased significantly and varied between 0.32 and 0.37% (Figure 20 C). This is likely explained by the high applications of S with algae fibre; 120 mg per pot.

Sodium is not an essential plant nutrient and has been classified as a functional plant nutrient (Subbarao et al., 2003). The Na concentration in unfertilised plants was 0.49% of DM, which was comparable to levels obtained with Calcinit (Table 8). It increased to an average of 0.58% of DM with the application of fish bones (Table 8). With algae fibre, the values decreased significantly to 0.17 - 0.24% of DM. Low application of algae fibre showed lower values than with high application.

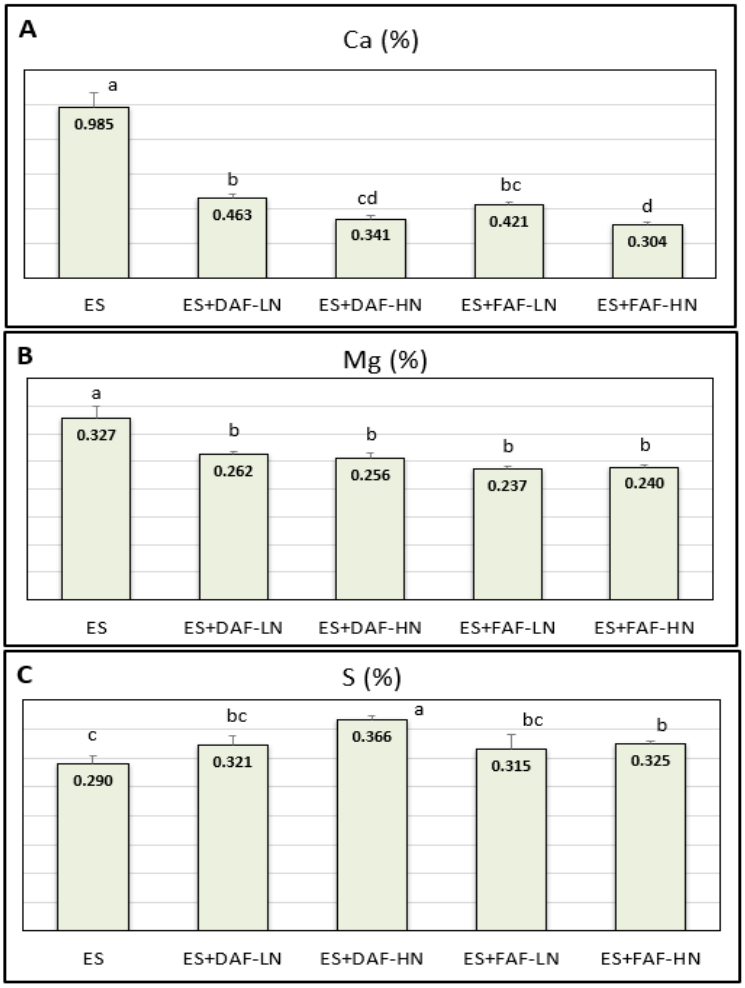


Figure 20. Concentrations of P, K, S and Ca (% of DM) in above-ground plant material of ryegrass grown on unfertilised soil and soil amended with algae fibre (dried and fresh). Experimental soil (ES), soil amended with dried or fresh algae fibre (DAF or FAF), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above bars indicate statistically significant differences (Tukey t-test, P = 0.05).

3.6.2 Micronutrients

In comparison to the control plants, all fertilised treatments had lower concentrations of Fe (Table 9). The Fe concentration in control was 0.116 mg/g of DM, whereas the lowest value, 0.071 mg/g DM, was observed with fresh algae fibre. B concentrations were not much affected by fertilisation and ranged from 0.010 to 0.012 mg/g DM (Table 9), except from algae fibre where the values ranged from 0.016 to 0.017. For Cu and Mo concentrations, there was not much effect of fertilisation and values ranged from 0.006 to 0.009 mg/g DM for Cu, and 0.001 to 0.003 mg/g DM for Mo. Similar to what was found for Fe, the concentrations of Mn were lowest in plants amended with algae fibre (0.060 to 0.077 mg/g DM), as compared with 0.145 mg/g DM in the control (Table 9). The concentrations of Zn were similar for the control (0.041 mg/g DM), and plants amended with Calcinit (0.039 and 0.041 mg/g DM). Plants amended with fish bones and algae fibre showed slightly lower values, ranging from 0.032 to 0.038 mg/g DM (Table 9).

Table 9. Concentrations of micronutrients (mg per g DM) in above-ground material of ryegrass.

Sample Code	Fe	B	Cu	Mn	Zn	Mo
ES	0.116	0.012	0.008	0.145	0.041	0.002
ES+DFB-LN	0.095	0.010	0.008	0.124	0.037	0.001
ES+DFB-HN	0.086	0.011	0.008	0.118	0.036	0.001
ES+FFB-LN	0.088	0.011	0.008	0.127	0.038	0.002
ES+FFB-HN	0.092	0.011	0.007	0.110	0.035	0.001
ES+DAF-LN	0.084	0.017	0.007	0.066	0.032	0.003
ES+DAF-HN	0.078	0.017	0.009	0.060	0.036	0.003
ES+FAF-LN	0.071	0.016	0.007	0.072	0.034	0.003
ES+FAF-HN	0.073	0.016	0.006	0.077	0.035	0.003
ES+CAL-LN	0.090	0.011	0.009	0.135	0.039	0.002
ES+CAL-HN	0.078	0.011	0.008	0.125	0.041	0.001

Mean values (n = 4) of accumulated aboveground plant material, stubble included. B Boron, Cu Copper, Fe iron, Mn Manganese, Mo Molybdenum, Zn Zinc. Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN).

3.6.3 Trace elements and potentially toxic elements

The concentrations of potentially toxic elements As and Hg were below the limit of detection in all treatments (Table 10), which was for As < 0.0005, and for Hg < 0.0001 mg/g DM. Since the concentration of As in algae fibre was quite high (33 mg/kg DM; Table 5), this was an exciting result. The concentration of Pb was near the limit of detection, < 0.0002 mg/g DM, except for a few treatments. For Cr, the concentrations were quite similar across all treatments. The concentrations of Cd varied from < 0.0004 to 0.00015 mg/g DM, being lowest in plants amended with algae fibre and highest in plants given Calcinit (Table 10). The concentrations of Co also varied from below the limit of detection < 0.0002 to 0.0006 mg/g DM, being highest in plants amended with algae fibre. Concentrations of Al and Ni were lower in plants fertilised with algae fibre (Table 10). Selenium is an important essential nutrient which often is scarce in Norwegian soil. Plants amended with algae fibre had somewhat higher concentrations of this nutrient.

Table 10. Concentrations of trace elements / heavy metals (mg/g DM) in ryegrass above-ground plant material (n = 4).

Trace element	ES	ES+DFB-LN	ES+DFB-HN	ES+FFB-LN	ES+FFB-HN	ES+DAF-LN	ES+DAF-HN	ES+FAF-LN	ES+FAF-HN	ES+CAL-LN	ES+CAL-HN
Se	0.0003	< 0.0002	< 0.0002	< 0.0002	< 0.0002	0.0004	0.0006	0.0005	0.0006	< 0.0002	< 0.0002
Co	< 0.0002	< 0.0002	< 0.0002	< 0.0002	< 0.0002	0.0004	0.0006	0.0005	0.0006	< 0.0002	< 0.0002
Al	0.0546	0.0224	0.0171	0.0180	0.0259	0.0243	0.0168	0.0143	0.0137	0.0301	0.0132
Ni	0.0012	0.0013	0.0012	0.0012	0.0012	0.0010	0.0007	0.0008	0.0007	0.0013	0.0012
Cr	0.0029	0.0030	0.0025	0.0030	0.0026	0.0031	0.0028	0.0028	0.0030	0.0027	0.0026
Cd	0.00012	0.00012	0.00011	0.00011	0.00011	0.0008	0.0006	0.0007	0.0004	0.00015	0.00014
Pb	< 0.0002	0.0003	0.0002	< 0.0002	0.0002	0.0002	< 0.0002	< 0.0002	< 0.0001	< 0.0002	< 0.0002
As	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
Hg	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Mean values (n = 4) of accumulated aboveground plant material, stubble included. Se Selenium, Co Cobalt, Al Aluminium, Ni Nickel, Cr Chromium, Cd Cadmium, Pb Lead, As Arsenic, Hg Mercury. Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN).

3.7 Effects of fertilisation on plant nutrient uptake

The uptake of plant nutrients (mg per pot) from untreated and fertilised soil was calculated by multiplying the concentrations (section 3.6) by the total amount of above-ground plant material (stubble included), and are presented in Tables 11 - 13. Statistically significant effects are shown in Figures 21 - 24.

3.7.1 Macronutrients

The N uptake in above-ground plant material varied from 65.1 mg per pot in the control treatment to 123.8 mg per pot in plants fertilised with the high amount of dried fish bones (Table 11). In general, the N uptake increased by N fertilisation. Fertilisation with fish bones having high N significantly enhanced N uptake compared to the control plants (Figure 21A). With algae fibre, the N uptake also increased with higher applications but was not statistically different from the control (Figure 22A).

Table 11. Uptake of macronutrients (mg per pot) in above-ground plant material of ryegrass.

Sample Code	N	P	K	Ca	Mg	S	*Average Total DM (g)*
ES	65.1	5.7	46.7	21.2	7.0	6.2	*2.15*
ES+DFB-LN	103.6	10.2	52.3	30.0	10.0	7.1	*2.98*
ES+DFB-HN	123.8	18.0	50.2	43.0	14.0	8.4	*3.62*
ES+FFB-LN	98.6	12.5	53.8	32.1	10.7	8.2	*3.09*
ES+FFB-HN	117.6	16.0	46.8	36.1	12.8	7.6	*3.41*
ES+DAF-LN	61.5	7.9	119.5	11.5	6.5	7.9	*2.47*
ES+DAF-HN	79.3	10.8	156.2	10.4	7.8	11.1	*3.04*
ES+FAF-LN	54.1	8.1	110.0	9.2	5.2	6.8	*2.18*
ES+FAF-HN	60.0	10.9	135.6	7.8	6.1	8.3	*2.55*
ES+CAL-LN	79.5	5.3	45.4	29.6	8.1	5.9	*2.43*
ES+CAL-HN	92.7	5.4	40.0	35.0	8.5	5.6	*2.74*

Mean values (n = 4) of plant uptake in accumulated aboveground plant material, stubble included. Ca Calcium, N Nitrogen, P Phosphorous, K Potassium, Mg Magnesium, S Sulphur. Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN).

The uptake of P in control plants was 5.7 mg per pot (Table 11). Following a similar trend as was found for N uptake, P uptake also increased with the level of fertilisation, except from plants amended with Calcinit, where the uptake was similar at both N levels (5.3 mg per pot for low and 5.4 for high levels of N). Plants amended with fish bones had a very high P uptake, ranging from 10.2 to 18.0 mg per pot (Figure 21 B, Table 11). Since the levels of total P were high in fish bones (120.0 g per kg DM, Table 4), this was not an unexpected outcome. Plants amended with algae fibre also had a significantly higher P uptake than the control with both low and high applications, ranging from 7.9 to 10.9 mg per pot (Figure 22 B, Table 11). This shows that even if the P concentration in the algae fibre was not very high (2.4 - 2.5 g per kg DM as compared with 120 g in fish bones, Table 4), it was enough to support plant growth and uptake of P.

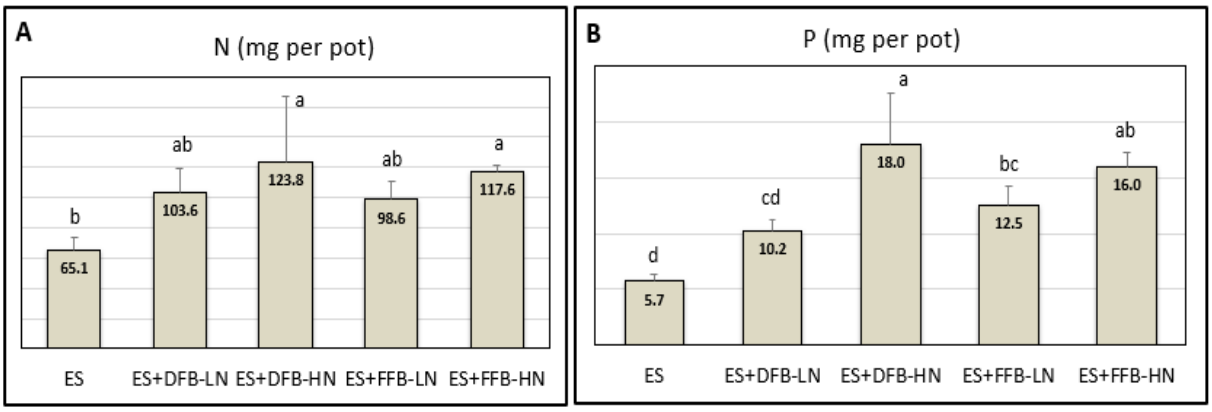


Figure 21. Uptake of N and P (mg per pot) in above-ground plant material of ryegrass grown in unfertilised soil and soil amended with fish bones (dried and fresh). Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above bars indicate statistically significant differences (Tukey t-test, P = 0.05).

The K uptake was 46.7 mg per pot in the control treatment (Table 11). With fish bones, the values were comparable, ranging from 46.8 to 53.8 mg per pot. Plants fertilised with Calcinit had a relatively lower K uptake, decreasing from 45.4 to 40 mg per pot with the low and high application. Algae fibre significantly enhanced K uptake, and the uptake increased with fertilisation level (Figure 22 C). With low application, K uptakes were 110 and 120 mg per pot, and with a high application they were 136 and 156 mg (Figure 22 C). This result corresponds well with the high concentration of total K in algae fibre (130.0 g per kg DM, Table 4). If sufficiently large amounts of easily available K are present, then uptake of K can be exaggerated by the tendency of plants to take up much more K than they require, which is known as luxury consumption (Weil and Brady, 2016). This may hamper the uptake of less mobile, divalent cations such as Ca and Mg.

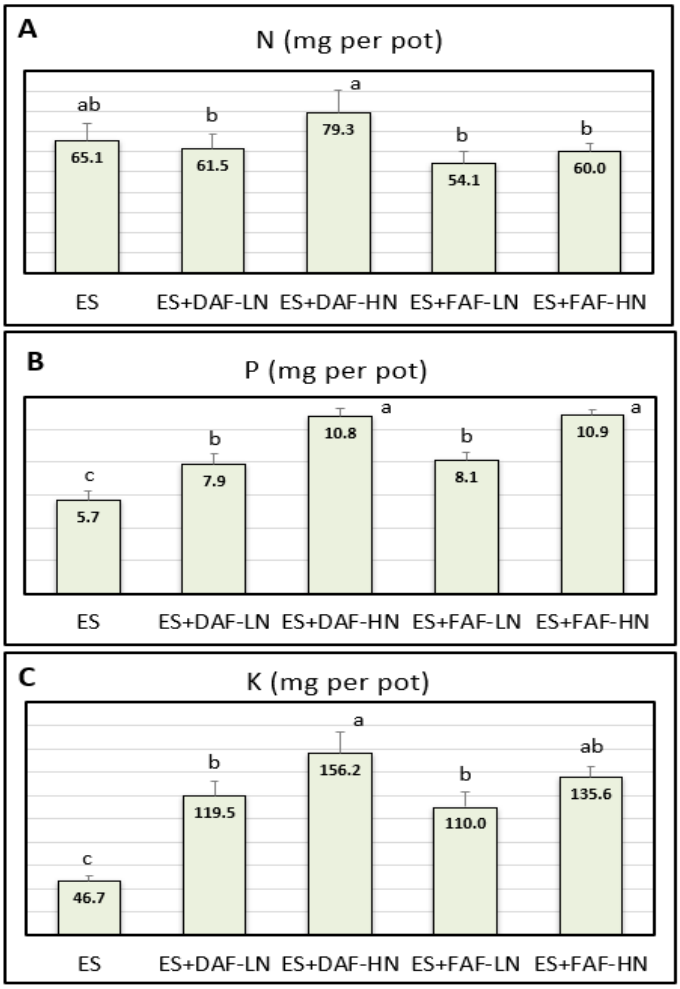


Figure 22. Uptake of N, P and K (mg per pot) in above-ground plant material of ryegrass grown on unfertilised soil and soil amended with algae fibre (dried and fresh). Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), with low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above bars indicate statistically significant differences (Tukey t-test, P = 0.05).

The uptake of Ca in the control plants was 21.2 mg per pot (Table 11). With Calcinit, this value increased to 30 and 35 mg per pot with low and high fertilisation levels. Fish bones also significantly enhanced the uptake of Ca compared with control (Figure 23 A), and more with a high application. Contrary to what was found for fish bones and Calcinit, amendment with algae fibre showed reduced uptake of Ca, where the maximum reduction was observed with the application of high amounts of fresh algae fibre (Figure 24 A). Ca uptake in plants amended with algae fibre ranged from 7.8 to 11.5 mg per pot (Table 11). The concentration of Ca in algae fibre was 68 g per kg DM (Table 4), and the soil Ca-AL was significantly increased with the application of this material (Figure 17 B). Hence, the low uptake of Ca by plants amended with algae fibre is somewhat surprising but may be explained by a luxury uptake of K as discussed above. Hence, application of algae fibre should be done with care, especially when growing feed for animals who need calcium for producing bones, milk, eggshells and more.

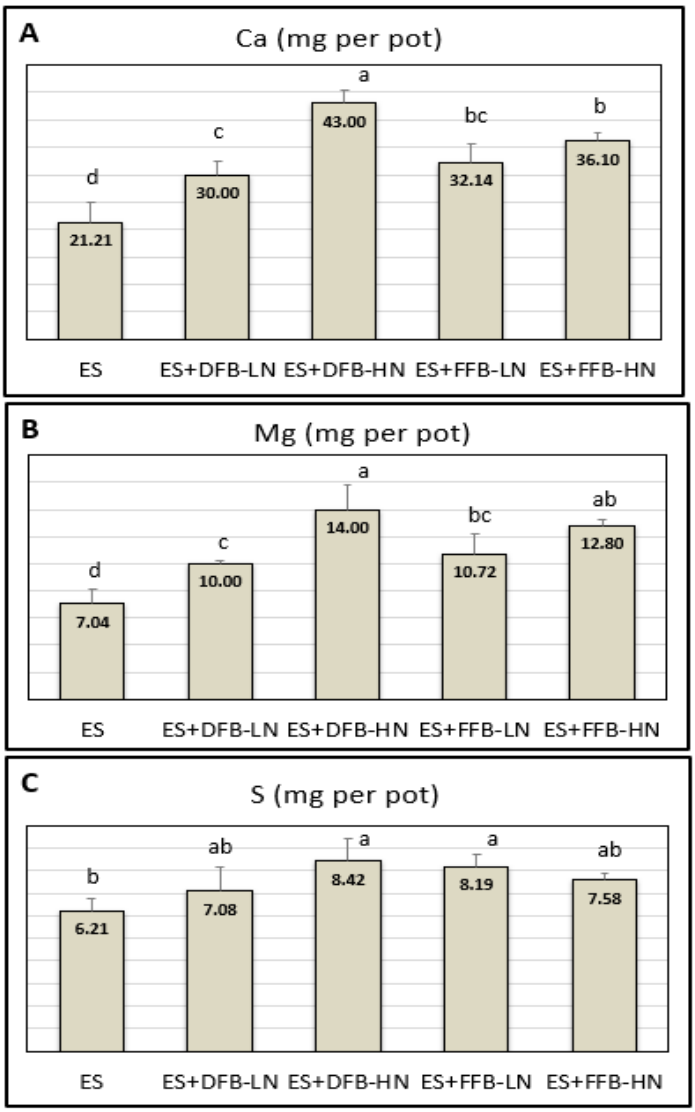


Figure 23. Uptake of Ca, Mg and S (mg per pot) in above-ground plant material of ryegrass grown on unfertilised soil and soil amended with fish bones (dried and fresh). Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), with low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above bars indicate statistically significant differences (Tukey t-test, P = 0.05).

The uptake of Mg in control plants was 7.0 mg per pot and increased by fertilisation with fish bones to 10 - 14 mg per pot (Table 11, Figure 23 B). Calcinit fertilisation also slightly increased Mg uptake to 8.1 and 8.5 mg per pot with a low and high application (Table 11). For algae fibre, there seemed to be somewhat better uptake of Mg from dried fibre than from fresh fibre (Figure 24 B), but in general, the uptake was not much higher than in the control in spite of reasonable concentrations of Mg in the fibre, 25 g per kg DM (Table 4). Again, this may be explained by a luxury uptake of K.

Na uptake was 10.5 mg per pot in above-ground plant material of control plants (Table 12). Na uptake in plants treated with Calcinit was slightly higher (about 12) than in the control. Na uptake increased with the application of fish bones, from 18 to 21 mg per pot, and decreased with application of algae fibre. The K uptake was very high in plants amended with algae fibre (Figure 22 C), and the low Na uptake in these plants is likely caused by a competition between K^+ and Na^+ since

K and Na ions compete for entry into plant cells due to their similar radius and ion hydration strategies (Zhang et al., 2009). Since Na concentrations in the fertilisers were not measured, this cannot be further assessed in this study.

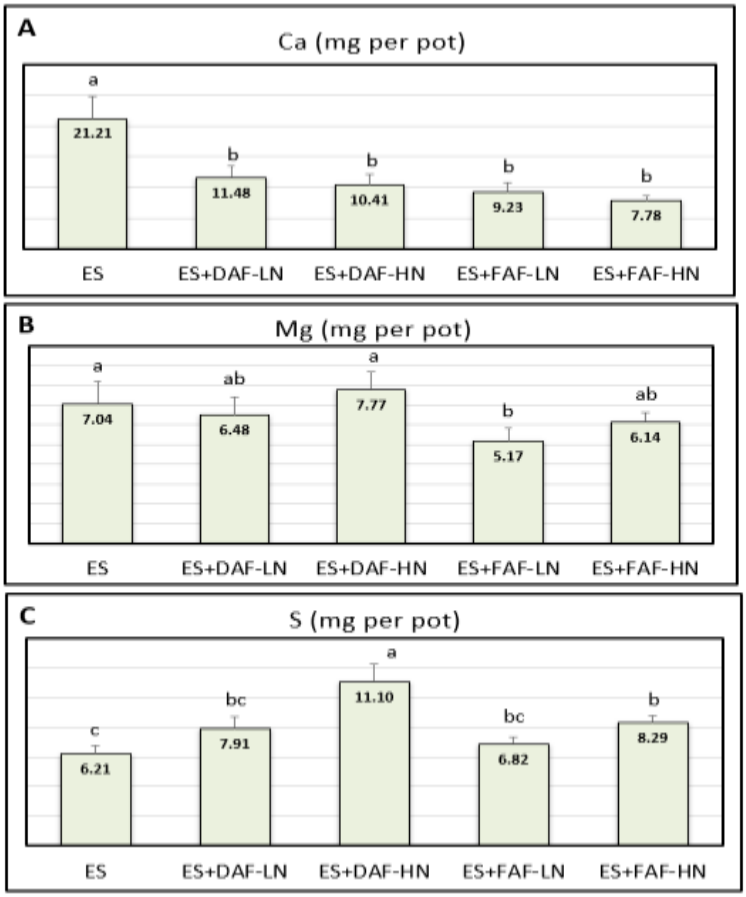


Figure 24. Uptake of Ca, Mg and S (mg per pot) in above-ground plant material of ryegrass grown on unfertilised soil and soil amended with algae fibre (dried and fresh). Unfertilised plants in experimental soil (ES), plants amended with dried or fresh algae fibre (DFB, FFB), low or high application of N (LN, HN). Error bars represent the means ± SD (n = 4). Different letters above the bars indicate statistically significant differences (Tukey t-test, P = 0.05).

Table 12. Uptake of sodium (mg per pot) in above-ground material of ryegrass.

Sample Code	Uptake	*Average total DM (g)*
ES	10.5 a	*2.15*
ES+DFB-LN	18.0	*2.98*
ES+DFB-HN	19.3	*3.62*
ES+FFB-LN	17.1	*3.09*
ES+FFB-HN	21.0	*3.41*
ES+DAF-LN	3.9 c	*2.47*
ES+DAF-HN	7.3 b	*3.04*
ES+FAF-LN	3.5 c	*2.18*
ES+FAF-HN	4.4 c	*2.55*
ES+CAL-LN	11.7	*2.43*
ES+CAL-HN	12.0	*2.74*

Mean values (n = 4) of plant uptake in accumulated aboveground plant material, stubble included. Unfertilised pants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN). Different letters in brackets indicate statistically significant differences (Tukey t-test, P = 0.05).

3.7.2 Micronutrients

The uptake of several micronutrients is affected by soil pH, and pH levels above 6 in sandy soils may decrease the uptake of Mn, which is the most sensitive micronutrient governed by soil pH (Scheffer and Schachtschabel, 1982). Also, the uptakes of Cu, Zn and Fe may be restricted by too high pH in soil (Marschner, 1995). Application of algae fibre in this study increased soil pH from 5.3 to 6.2 with low fertilisation level, and 6.8 with high level (Figure 12, section 3.5.1). Concentrations of Cu and Zn were comparable in algae fibre and fish bones, whereas algae fibre had much more B, Fe and Mn (Table 5, section 3.1). For Mo and Co, the concentrations in both materials were below the limit of detection.

The uptake of Fe was 0.238 mg per pot in above-ground plant material of control plants (Table 13). It was slightly lower (0.213 and 0.222 mg per pot with low and high N) in plants that were given Calcinit. With fish bones, the uptake of Fe was slightly higher, ranging from 0.284 to 0.314 mg per pot. For algae fibre, where much more Fe was applied, the uptake was lower and ranged from 0.153 to 0.237 mg per pot with slightly lower uptake in plants amended with fresh algae fibre. However, the uptake increased with the level of applied fertiliser and hence with increasing soil pH.

The uptake of B, Cu and Mn did not vary between the control and plants fertilised with Calcinit (Table 13). The uptake of B was significantly enhanced with high applications of fish bones and algae fibre. Cu uptake also increased significantly with high applications of fish bones, but with algae fibre, Cu uptake was significantly enhanced only with high application of dried material. For Mn and Zn uptake, higher values were observed with fish bones (Table 13). The uptake of Mn was lowest in plants amended with algae fibre, whereas for Mo, the uptake increased with algae fibre.

Table 13. Uptake of micronutrients (mg per pot) in above-ground material of ryegrass.

Sample Code	Fe	B	Cu	Mn	Zn	Mo	*Average total DM (g)*
Experimental soil in comparison to fish bones fertilisation							
ES	0.238	0.025 c	0.018 c	0.309 b	0.088 b	0.004	*2.15*
ES+DFB-LN	0.284	0.029 bc	0.023 bc	0.369 ab	0.110 ab	0.004	*2.98*
ES+DFB-HN	0.310	0.041 a	0.030 a	0.427 a	0.133 a	0.004	*3.62*
ES+FFB-LN	0.272	0.034 abc	0.023 bc	0.390 ab	0.118 ab	0.005	*3.09*
ES+FFB-HN	0.314	0.037 ab	0.025 ab	0.376 ab	0.120 ab	0.004	*3.41*
Experimental soil in comparison to algae fibre fertilisation							
ES	0.238	0.025 c	0.018 b	0.309 a	0.088 b	0.004 b	*2.15*
ES+DAF-LN	0.209	0.041 ab	0.016 b	0.161 b	0.079 b	0.008 a	*2.47*
ES+DAF-HN	0.237	0.052 a	0.028 a	0.182 b	0.109 a	0.009 a	*3.04*
ES+FAF-LN	0.153	0.035 bc	0.015 b	0.156 b	0.074 b	0.007 a	*2.18*
ES+FAF-HN	0.186	0.042 ab	0.016 b	0.197 b	0.088 b	0.008 a	*2.55*
Experimental soil in comparison to Calcinit fertilisation							
ES	0.238	0.025	0.018	0.309	0.088 b	0.004	*2.15*
ES+CAL-LN	0.222	0.028	0.023	0.326	0.095 ab	0.004	*2.43*
ES+CAL-HN	0.213	0.029	0.022	0.342	0.113 a	0.004	*2.74*

Mean values (n = 4) of plant uptake in accumulated aboveground plant material, stubble included. Unfertilised plants in experimental soil (ES), plants amended with dried or fresh fish bones (DFB, FFB), plants amended with dried or fresh algae fibre (DAF, FAF), plants amended with Calcinit (CAL), low or high application of N (LN, HN). Different letters indicate statistically significant differences (Tukey t-test, P = 0.05).

4 Conclusions

- Fertilisation of ryegrass with fish bones gave more rapid plant growth, and higher yields of above-ground dry matter (DM) compared with algae fibre and mineral fertiliser Calcinit. Nitrogen (N) was applied in a higher amount with Calcinit than with fish bones, but the yields were still considerably higher with fish bones. This is likely due to the high concentration of phosphorus (P) in the fish bones.
- The utilisation of N in the fertilisers was much better for fish bones than for Calcinit. This may show that soil P concentrations were limiting the plant growth in this soil so that applied N may not be utilised if no P is applied.
- Drying of algae fibre and fish bones at 105 °C did not affect negatively on the growth effects of these materials applied as fertilisers to ryegrass.
- Algae fibre had a high pH (10), which increased soil pH from initially 5.3 to 6.2 with low and 6.8 with high application of fertiliser. This may be beneficial in acidic soil but may affect the uptake of plant nutrients where the uptake is affected by soil pH.
- Application of algae fibre caused a luxury uptake of K in above-ground material of ryegrass, causing a low uptake of Ca, Mg and Na in plants fertilised with algae fibre.
- Concentrations of arsenic (As) in the algae fibre was high; 33 mg/kg DM. In spite of this, the concentrations of As in ryegrass plants were below the limit of detection.
- Both materials, fish bones and algae fibre, contain valuable plant nutrients and organic matter, which may have a positive effect as fertilisers and soil amendments. However, they are not well balanced with respect to the needs of agricultural crop plants. Hence, they need to be combined, with each other or with other sources of nutrients and organic matter, to produce a balanced fertiliser which fulfils the demand for crop plants.
- Further studies are required to possibly develop a commercial fertiliser product for organic growing from marine residual materials such as fish bones and algae fibre. Arsenic and cadmium in seaweeds need attention, as well as sodium. Algae fibre contains high concentrations of potassium, which may easily lead to luxury uptake, decreasing the uptake of calcium and magnesium with potentially negative health effects in animals.

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FrontPage Picture: Image taken by Anne de Boer, NIBIO. The picture shows close-up of fish bones particles surrounded by roots and experimental soil.