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Title:

Waste recovered by-products can increase growth of grass-clover mixtures in low fertility soils and alter botanical and mineral nutrient composition.

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Short title: Recycling by-products to grass - clover mixtures

Waste recovered by-products can increase growth of grass-clover mixtures in low fertility soils and alter botanical and mineral nutrient composition

Abstract

The effectiveness of four by-products (biogas digestate, pot ale, rockdust and wood ash) as fertilisers of a perennial ryegrass (*Lolium perenne* L.) – red clover (*Trifolium pratense* L.) mixture in terms of biomass production, botanical composition and macro- and micronutrient concentrations was tested in an outdoor pot trial. This was carried out over two growing seasons using two inherently low-fertility soils used for forage production. Macro- and micronutrients (N, P, K, Ca, Mg, Co, Cu, Mn, Mo and Zn) relevant for crops and livestock were determined in soils and plants. All the by-products increased overall biomass production and affected nutrient concentrations of the individual plant species to varying degrees. In addition the competitive balance between grass and clover was altered leading to different botanical composition in the different treatments and consequently differences in the nutrient concentrations of the species mixture. Changes were due to the nutrients applied in the by-products per se and/or to changes in the soil chemistry caused by the by-products. The results suggest a potential to enhance agricultural productivity through improved production and quality of forage on less fertile land by matching of by-products and soil properties.

Keywords: botanical composition, by-product recycling, crop growth, crop quality, macro- and micronutrient concentrations

Introduction

The efficiency of using natural resources in crop production systems is an active area of research to meet needs both at farm level and for society in general. This includes the more efficient use of renewable plant nutrient resources found in recovered by-products as this has capacity to decrease Global Warming Potential and accumulated energy use in arable farming systems (Tuomisto *et al*., 2012). To be useful as fertilisers, these should provide suitable contents of plant-available macro- and micronutrients, and improve soil physical, chemical and biological conditions whilst minimizing transfers of deleterious elements or compounds to the soil. There is an increasing range of recovered by-products considered for use as soil amendments with widely varying composition especially in relation to micronutrients. However, the efficiency of these materials as fertilisers and possible side-effects of their use at rates corresponding to crop needs and long-term nutrient balance in the receiving fields are often lacking. Instead, investigations have often focussed on the potential negative effects of by-product disposal at application rates higher than agronomic requirements (e.g. Bucknall *et al*., 1979; Etiegni *et al*., 1991; Krejsl and Scanlon, 1996), or on short-term effects such as shoot scorch after application in growing crops (Naylor and Shortreed, 1981).

Permanent and temporary grasslands, used as pastures or for ley production, are important land uses and cover large areas around the globe; e.g. in 2007 permanent grassland covered 33% of the agricultural area in Europe (Eurostat, 2012). Grasslands can show high production capacity even on less fertile land and under low or moderate fertiliser nitrogen (N) input, provided that they contain N_2 fixing legumes. To realise this potential in full, macro- and micronutrient availability must be inherently sustainable or augmented on a regular basis. The less productive agricultural lands often used for forage production in Northern Europe tend to have low inherent nutrient supplying capacity through soil mineral weathering and thus require inputs of macro- as well as micronutrients to sustain their fertility (Edwards *et al*., 2012). Lower productivity coupled with increasing fertiliser costs significantly limits the economic margins from such land. Furthermore, most studies on fertilisation including amendments have been carried out on productive agricultural soils such that knowledge is lacking on their effectiveness on less-fertile soils.

On less fertile land legume-containing leys (temporary grasslands in crop rotations) play an important role providing high quality forage. Requirements regarding feed digestibility, crude protein concentration and energy value are strict in high-productivity animal production, and in particular for high-yielding dairy cows. Furthermore, in addition to macronutrients, micronutrients such as essential cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn) as well as beneficial molybdenum (Mo) and nickel (Ni) are important components of good ruminant nutrition (Suttle, 2010); and concentrations of these nutrients are often higher in clover than in grasses (e.g. Pirhofer-Walzl *et al*., 2011; Lindström *et al*., 2013).

Mixtures of grasses and legumes often produce forage of higher digestibility and protein content compared to grass-only (Sleugh *et al*., 2000; Bertilsson and Murphy, 2003), lead to higher feed intake (Bertilsson and Murphy, 2003) and - unless the leys are heavily N fertilised - give more even biomass production across the growing season compared with pure grass leys (Sleugh *et al*., 2000). Hence the balance between grasses and legumes such as clover is an extremely important objective in managing pastures. Species mixtures result invariably in more complex crop responses to fertilisation or soil amendments because the individual species may react quite differently due to their differing nutritional requirements and tissue concentrations (Cope and Rouse, 1977; Lindström *et al*., 2013). It is a significant challenge therefore to increase production levels of mixed stands while maintaining the desired botanical composition of the ley and also to evaluate experimental results from them. However, as these forage species are frequently intercropped it is essential to carry out an evaluation of fertiliser effects in mixed stands.

The objective of the present study was to evaluate the fertiliser value of selected organic and inorganic by-products in terms of their ability to increase biomass production and affect the mineral composition of forage crops (mixtures of red clover and perennial ryegrass) on low-fertility soils. The following specific hypotheses were tested: 1) the by-products can all be used to improve the bioavailability of both macro- and micronutrients to plants by adding additional nutrients and altering soil properties; 2) as a consequence, overall crop growth will increase with addition of amendment, but the differing nutrient and pH preferences of the individual species will lead to different botanical compositions; 3) wood ash and rockdust add plant-available nutrients (except N) and increase soil pH; 4) pot ale liquor specifically increases Cu availability, uptake and plant concentration; 5) biogas digestate adds a balanced mix of plant-available nutrients reflecting the composition of the biogas feedstock. To test these hypotheses one soil was selected from each of two agricultural areas with coarse textured soils derived from nutrient-poor parent material. In order to avoid effects of differing weather and drainage conditions on the nutrient uptake and composition of the plants (Roche *et al*., 2009), these soils were used in an outdoor pot experiment where these conditions could be kept equal. In order to understand the plant-soil-amendment interactions, we grew a model grass/clover mixture but have assessed growth and nutrient uptake separately for each species.

Materials and methods

A pot experiment with a completely randomised design with 4 replications was established in summer 2009 and treatment effects determined the following year. To ensure relevance to farmers´ practice, application rates conformed to national regulations and guidance (Swedish Environmental Protection Agency, 1994 and Swedish Board of Agriculture, 2004). A mixture of red clover (*Trifolium pratense* L., cv. Nancy) and perennial ryegrass (*Lolium perenne* L., cv. Helmer) was used as a simplified model of the mixed ley stands in common use. Top soils with low nutrient status were used (Table 1) as representatives of soils derived from highly siliceous parent material that is common for Northern European grassland soils. Hollsby (59°48'N, 13°31'E) is a postglacial silt loam originating from mainly granitic and sandstone bedrock and used for grazing on semi-natural grassland (Table 2). Rådde (57°36'N, 13°15'E) is a till with sandy loam texture developed from gneissic and granitic parent material and used for ley production.

Treatments

The by-products were selected based on their relative concentrations of macro- and micronutrients and non-nutrient elements and an assessment of potential volumes available to the agricultural sector. They included a volcanic (pyroxene-andesite) rockdust (Cameron *et al*., 2010; Ramezanian *et al*., 2013) as sole amendment or in combination with N, bottom ash from mixed deciduous wood as sole amendment or in combination with N, a whiskey distillery by-product (pot ale) and biogas digestate from a biogas plant fed with source separated household waste and grass silage (Table 3). A fully fertilised treatment in split applications (in spring and after harvest 1 and 2; Table 3) and an unamended control were included for comparison. As a general rule the by-products were applied at the maximum allowable 7-year application rates of nutrient and non-nutrient elements as stated by the Swedish Environmental Protection Agency (1994) for trace elements (cadmium (Cd), chromium (Cr), Cu, mercury (Hg), Ni, lead (Pb), and Zn), and the Swedish Board of Agriculture (2004) for P. However, to maintain a desirable proportion of clover the N application rate via biogas digestate and pot ale was set at 150 kg total N ha^{-1} equivalent. On the other hand, low plant availability of elements was anticipated for the rockdust applications; hence the trace element limitation prescribed by Swedish Environmental Protection Agency was exceeded (for Ni, Cr) and a higher application rate as recommended by the rockdust supplier was used. The mineral N application rate in the fully fertilised treatment and rockdust+N and wood ash+N treatments was similarly set to achieve a balanced mix of grass-clover in the pots. All by-products (and the full fertilisation) were applied before establishment of the experimental crop in the summer 2009. In spring 2010 and after the first harvest, macronutrients were again added to the fully fertilised treatment and N was added in the rockdust+N and wood ash+N treatments. The first and second harvest results suggested NPK were still the major limiting factors to the evaluation of the by-products. Hence, after the second harvest we augmented with NPK to evaluate separately the effects from other component nutrients; however, rockdust-N and wood ash-N treatments did not receive any additional N.

Establishment, growth conditions and samplings

Both soils were sieved through an 8×18 mm aluminium mesh and thoroughly homogenised before use in the pot experiment. At establishment, fresh soil (corresponding to 6 kg dry weight (DW)) was mixed with the respective amendments, transferred to each pot (220 mm inner Ø, 250 mm depth) and on 27 July 2009 sown with a mixture of red clover and perennial ryegrass which were thinned to 10 plants of each species per pot approximately two weeks after emergence. The pots were subsequently kept outdoors under semi-natural conditions in a netted, unroofed area and irrigated with deionized water as needed to complement precipitation. During the establishment year, plants were left intact for the entire growth period, and then overwintered in a climate chamber at -1°C to +1°C.

The plants were harvested 15 June, 20 July, and 20 August 2010 and at all three harvests the clover was between stem elongation and early flowering stage and the grass was at earing stage. Before harvest the plants were showered with deionized water to minimize plant contamination by dust. Plants were subsequently cut at 5cm above soil level with stainless steel scissors, sorted into clover and grass and dried in perforated plastic bags at 50˚C in a forced-ventilation dryer for at least 48 h. The plant samples were weighed and milled to a particle size below 1 mm using a cutting mill with a titanium knife (Grindomix GM 200, Retsch GmbH). Precautions to counteract trace element contamination (Dahlin *et al.*, 2012) were taken at all handling steps.
Soil sampling was carried out on 22-29 September 2010, all pots of one replicate per day. Ten cores were taken from each pot using a corer (9 mm inner Ø) to the pot´s full depth, and the samples air dried.

Chemical analyses

Soil samples, wood ash and rockdust, were a) digested in concentrated HNO_3, HCl and HF in closed Teflon containers in a microwave digestion system and Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, sulphur (S) and Zn measured on ICP-SFMS (sector technique), and b) fused with lithium metaborate, then dissolved in HNO_3 and aluminium (Al), Ca, K, magnesium (Mg), Mn and P measured by ICP-AES. Biogas digestate, pot ale and the plant material were digested in concentrated ultrapure HNO_3 and HF in open vessels in a microwave oven, followed by filtering, and measurement of all elements on ICP-SFMS.

Total N and C concentrations in plant and soil samples were analysed by high temperature induction furnace combustion using LECO CN2000 (LECO Corporation, St Joseph, MI, USA). Soil electrical conductivity (EC) was measured in a solution of deionized water and thereafter $CaCl_2$ was added (0.01 M) and pH determined (Sumner, 1994). EDTA extractable P, K, Ca, Mg, S, Co, Cu, Mn, Mo and Zn were analysed after extraction according to Streck and Richter (1997) although Na-EDTA was used and the extracts were analysed by ICP-MS ELAN 6100 DRC (Perkin Elmer SCIEX, Waltham, MA, USA). EDTA extractable concentrations are reported as Ca_{EDTA}, Co_{EDTA}, etc.

Certified reference material (NIST Wheat Flour, National Institute of Standards and Technology, Gaitersburg, MD, USA) was included in all batches for plant material analysis. For soil analyses an in-house standard was included in each batch.

Soil particle size distribution was determined according to ISO 11277:1998 (ISO, 1998), and cation exchange capacity (CEC) and base saturation (BS) calculated from Parker (1929) and Thomas (1982). Phosphorus, K, Ca, and Mg were extracted with ammonium lactate/acetic acid (AL) solution (Swedish Standards Institute, 1993) for comparison with Swedish soil maps. The mineralogical composition of < 2mm soil was determined by XRD on spray dried random powder samples (Hillier, 1999) and quantitative analyses done using a full pattern fitting method (Omotoso *et al*., 2006).

All-season average concentrations of the nutrients taken up by the plants were calculated by weighting of biomass DW at each harvest, and overall nutrient concentrations of the species mixtures calculated by taking into account the botanical proportion of each species in each mixture. The nutrient off-take was also calculated, here defined as the total amount of macro- and micronutrients removed with the total (i.e. summed grass and clover) biomass harvested per unit area.

Statistical analysis

Statistical analysis was done using the JMP 10.0.0 (SAS Institute Inc., Cary, NC, USA) two-factorial variance analysis including by-product/fertiliser treatment and soil type in the data. When needed, data were transformed to the natural logarithm or square root to achieve normal distribution of residuals. Multiple linear regressions followed by pairwise correlations were performed using the Holm (1979) method to control the family-wise error of the multiple regressions; plant biomass accumulation was tested vs plant concentrations of all measured nutrients, and vs soil pH; plant concentrations of all nutrients were tested vs soil pH; and plant concentrations were tested vs the respective EDTA extractable soil concentrations. All differences described in the text are significant at $p<0.05$.

Results

Soil response to amendment

The pH range across all treatments was 4.6-5.4 (Hollsby) and 4.4-5.4 (Rådde) (Table 4). Across soils, pH was significantly higher after wood ash application (average pH 5.2) than in the unamended control and most other by-product amended soils (average pH 4.9). The EC was similar in all by-product amended and non-amended soils but higher in the fully fertilised treatment (Table 4).

The wood ash amended soils had higher EDTA extractable concentrations of Ca, K and Mg compared with other treatments but low-to-average concentrations of other nutrients (Table 4). The pot ale treatment had the highest Cu_{EDTA}. The fully fertilised treatment frequently had higher than average EDTA extractable concentrations, but had the lowest average Cu_{EDTA} on the Hollsby soil.

Plant biomass harvested

Grass growth was strong during spring but subsequently declined, whereas the opposite was true for the clover (data not shown). The additional fertilisation with N, P and K after the second harvest did not significantly increase growth of either clover or grass, except that of clover on the rockdust amended Rådde soil. However, on average this fertilisation more than doubled the plant K concentration and to a smaller degree increased the P concentration in most of the treatments while it decreased the plant Mg concentration compared to the second harvest. Nevertheless, as the overall treatment effects were similar at each harvest, data are presented averaged across the growing season.

The harvested plant biomass was strongly affected by the amendments (Fig. 1A and B), with higher grass biomass on soils amended with by-products containing plant available N or receiving mineral N (fully fertilised, pot ale, and wood ash and rockdust in combination with N). However, treatments with high grass biomass generally had a relatively small clover biomass (except for the fully fertilised treatment.) This was most clearly seen where mineral N was applied along with wood ash and rockdust as compared to when wood ash and rockdust were added alone. The total biomass production subsequently varied less, but was significantly higher on all amended soils compared with the unamended control and particularly high on the fully fertilised soil. As a result of the different response of clover and grass to the by-products and the mineral fertilisation, the botanical composition of the species mixture differed widely from 20-50% clover in the wood ash+N, rockdust +N and fully fertilised treatments to around 75% clover in the wood ash and rockdust amended treatments (Fig. 1A and B).

Clover and grass nutrient concentrations

Plant nutrient concentrations in the unamended control was frequently low indicating that more than one nutrient may have restricted growth. Clover concentrations were at or below reported critical concentrations of K (both soils), Mg (Rådde) and P (Rådde), and close to reported critical concentrations for N (both soils) and P (Hollsby) (Supp. 1A and B). Grass concentrations of K (both soils), N (both soils) and P (Rådde) were below critical concentrations, and close to critical concentrations for Cu (Hollsby), Mg (both soils) and P (Hollsby) (Supp. 2A and B). From a fodder perspective, the species mixtures were below recommended concentrations for Co (Rådde), Cu (Rådde), N (both soils) and Zn (Hollsby), and close to the lowest recommended concentrations for K (both) and Mg (Rådde) (Supp. 3A and B).

Amendment and fertiliser applications lead to lower clover concentrations of Cu and N on both soils and P on Hollsby soil. Clover from the wood ash treatment generally had among the lowest concentrations of Ca, Co, Cu and N, for both soils (Fig. 2A and B). The fully fertilised treatment generally showed low clover concentrations of Cu and Mo, but high K, Mg, Mn and N. The biogas digestate, pot ale, and rockdust (with or without N) generally showed intermediate nutrient concentrations. When wood ash or rockdust application was combined with N, clover concentrations of Ca, Mg and Mo were significantly higher than when grown without N, and for rockdust also Co and Mn were higher. There was also a tendency for lower K concentrations in both wood ash and rockdust amended soils when N was added.

After by-product amendment or fertilisation, grass Cu and Ca concentrations were lower on both soils and Mg, Mo and P concentrations on the Hollsby soil (Fig. 3A and B). In the wood ash and rockdust treatments, Ca, Co and N were higher and K lower when N was applied, which differed from the effects on the clover.

Analysed across all treatments and both soils, clover had significantly higher concentrations of Ca (240%), Co (30%), Cu (140%), Mg (70%), Mo (5%), N (80%) and Zn (80%) than the grass, whereas concentrations were lower for K (30%) and Mn (50%) (Supps. 1A and B, and 2A and B). Phosphorus concentrations were similar and not significantly different between species. On both soils, K in clover and grass was low in relation to reported plant needs, and P and Cu concentrations were also similarly low in relation to plant needs in some treatments (Supps. 1A and B, and 2A and B). Magnesium concentrations apparently did not meet the plant demand in some treatments on the Rådde soil.

Overall nutrient concentrations and off-takes of the species mixture

Overall nutrient concentrations of the mixtures were strongly affected by the treatments, with a median ratio of 1.9 between the highest and lowest concentrations within each soil (Fig. 4a and b, Suppl. 3a and b). For example, Mg concentrations were higher on the rockdust and wood ash amended soils than on the ones amended by pot ale and digestate, Cu and Zn were generally high after digestate application, but Cu low after pot ale amendment, and Co, Mn and Zn low on the rockdust and wood ash amended soils. Application of N with the rockdust or wood ash generally decreased the overall concentrations of Ca, Cu, K and N on both soils and Mg on Hollsby soil.

The nutrient off-take with harvested biomass reflected nutrient concentrations as well as the biomass production of the clover and grass, respectively (Supp. 4a and b). The fully fertilised treatment thus generally showed the highest off-takes. The unamended control showed low off-takes for all nutrients, but Mn off-take was lowest from the wood ash and wood ash + N fertilised soils, and off-take of Cu was lowest from the pot ale and wood ash + N fertilised soils. Simultaneous application of N with the wood ash or rockdust produced significantly lower off-takes of Ca, Cu, Mg, K, and N compared to wood ash or rockdust only, but higher Mn off-takes for the rockdust fertilised soils.

Relations between soil characteristics, plant composition and growth

Amendment affected plant growth and nutrient concentrations depending on by-product composition and soil availability of the respective nutrients. Increasing soil pH generally was correlated with decreasing plant concentrations of Cu, Mn and Zn but with increasing plant Mo (both soils) and P (Hollsby) concentrations (Supp. 5). Biomass accumulation was, however, not significantly correlated with pH on either of the soils.

Correlations between plant nutrient concentrations and EDTA extractable soil concentrations were mainly with K, Mg and Zn (Supp. 5). Direct correlations between nutrient application rates and increased plant concentrations of the same nutrient were seen only for K (Hollsby clover *p*=0.0023; Rådde grass *p*=0.0003).

Clover biomass accumulation was inversely correlated with its Mg and Mo concentrations on the Hollsby soil (Supp. 5). Grass biomass was directly correlated with plant Mn and Zn concentrations but inversely correlated with plant Mo concentration. On the Rådde soil, grass biomass was directly correlated with plant Co, K, Mg and P concentrations.

Discussion

Testing the by-products in a pot experiment serves as a first step and needs to be followed by testing in the field. However, it gave the opportunity to test their effects on different soils under semi-controlled and equivalent conditions. The soils selected had low concentrations of K, Mg and several micronutrients compared with arable soils in Northern Europe (Reimann *et al*., 2000; Eriksson *et al*., 2010; Paterson, 2011; Swedish Monitoring Program, 2013). They thus served as representatives of soils commonly used for grazing and forage production and the potential for detecting any fertiliser effects of K, Mg and several micronutrients of the by-products was deemed to be good. Both soils had low pH, although not extreme for this type of land use. Nevertheless the low pH will have improved the availability of a majority of the micronutrients (Alloway, 2013), possibly contributing to sufficient plant uptake in spite of the low total soil concentrations of some of these nutrients.

Effects of by-product application on biomass production

Total biomass harvested was low on the unamended soils, much higher in the fully fertilised treatment, with the by-product amended treatments falling between these two extremes. This indicates that biomass production on the unamended soils was impeded primarily by nutrient supply. It was further evident that total biomass production was increased by all the by-products applied to these soils. Such increases have been reported for digestate-amended soils (Gunnarsson *et al*., 2010; Grigatti *et al*., 2011) and pot ale-amended soils (Douglas *et al*., 2003). This has also been reported for wood ash-amended soils (as reviewed by Demeyer *et al*., 2001), although application rates have often been considerably higher than agronomic requirements. Yield increases have also been reported after amendment with mafic rockdust (e.g. Kahnt *et al*., 1986; Bakken *et al*., 2000) although no yield effect was found by Campbell (2009) and Ramezanian *et al*. (2013) of the same type of andesitic rockdust as that used in the current experiment. The contrasting results highlight the importance of the original nutrient status of the soils for the scope of detecting nutrient supply from by-products, and also determine whether these products may potentially be useful as amendments to the respective soils. Rockdust may be most suitable therefore for soils with low capacity to supply plant nutrients, especially for K, Ca and Mg.

Clover and grass respond differently to treatments

The increased grass and decreased clover biomass production upon N fertilisation confirmed the increased competitiveness of perennial ryegrass vs. clover under high N availability and a shift in botanical composition frequently seen in swards under increased N fertilisation (Harris *et al*., 1996; Elgersma *et al*., 2000). However, by-products containing little or no N in this experiment (i.e. the wood ash and rockdust) also led to a shift in botanical composition compared to the unamended control, albeit with an increase in the proportion of the clover. Data from Ferreiro *et al*. (2011) also suggest that the white clover proportion in ash-amended leys increased relative to the unamended control in a similar manner, although this was not specifically tested in that study. The two organic by-products gave intermediate but distinctly different botanical composition. Consequently the agronomic management of clover in such low fertility soils needs to consider carefully the effect of amendments on the sward composition.

Both inherent soil properties and by-products affect grass and clover nutrient concentrations

The macro- and micronutrient concentrations of the grass and clover grown on the two soils was apparently affected by the inherent nutrient-supplying capacity of the soil, by the application of nutrients per se and indirectly through effects the amendments had on pH or the ionic composition of the soil solution. For example, the direct correlation between grass K concentration and harvested biomass on Rådde soil and a tendency towards a correlation on Hollsby soil, combined with the low plant concentrations suggests that K deficiency was limiting growth in some treatments on both soils. Potassium and Mg supply from the by-products was clearly indicated as both plant nutrient concentrations and off-takes increased, although to a differing extent; e.g. on average across the soils wood ash increased clover K and Mg concentrations by 55 and 35%, respectively, and total plant off-takes by 115% and 140%, respectively, compared with the unamended control. However, the results suggest K supply was still limited. Although Öborn *et al*. (2010) found a clear growth limitation only at a ryegrass K concentration of 1%, critical or sufficiency concentrations reported by Whitehead (2000) and Mengel (2007) suggest that grass as well as clover K concentrations were in the suboptimal range. The decreased K concentrations in the N-fertilised grass thus indicate a dilution effect (Jarrell and Beverly, 1981) through enhanced biomass accumulation which could not be matched by a corresponding increase in K uptake in spite of the K application via the wood ash or rockdust.

The correlation of plant nutrient concentrations or off-takes vs. EDTA-extractable soil concentrations was strong for Zn on both soils, for Mn on the Hollsby soil and K and Mg on the Rådde soil, but otherwise often weak, which illustrates that EDTA extraction is often only a crude estimate of plant available fractions of nutrients (e.g. Soriano-Disla et al., 2010). A particularly striking example of this was the EDTA extractable Cu concentration in the soils treated with pot ale which was highest of all treatments for both soils, but where the clover concentration and the total off-take were among the lowest. This indicates that Cu in the plant biomass not only was diluted due to increased growth (e.g. Reith *et al*., 1984; Kopsell and Kopsell, 2007), but also that the pot ale Cu was poorly available to the plants or other factors were reducing its uptake.

The availability of a number of micronutrients is known to be strongly affected by pH (Alloway, 2013). The pH increase from 5.0 to 5.4 in Hollsby and from 4.6 to 5.2 in Rådde could be expected to affect plant availability of nutrients in the wood ash and wood ash + N treatments. Clover and grass concentrations of Co, Cu, Mn and Zn were indeed low in these treatments whereas Mo concentrations were high. Other studies have also revealed decreased plant concentrations of micronutrients such as Cu and Mn (Krejsl and Scanlon, 1996) but increased plant Mo concentrations (Park *et al*., 2012) after wood ash application and these effects were attributed to an increased pH. However, in our experiment only off-takes of Mn were decreased compared with the control, suggesting that dilution due to increased biomass accumulation (Jarrell and Beverly, 1981) may have been a contributing factor for the decreasing plant concentrations at least for the remaining nutrients (Co, Cu and Zn).

Effects of botanical composition on plant mixture mineral concentrations

Enhanced N nutrition affected the species mixtures´ overall nutrient concentrations and off-take via effects on the botanical composition and nutrient concentrations of the individual species. Application of N with wood ash and rockdust increased plant concentrations of a majority of the macro- and micronutrients in the clover and/or the grass. Increased plant concentrations in response to N fertilisation were also reported by Fangmeier *et al*. (1997) for Ca, Mg, N, and Zn in wheat at the beginning of shoot elongation although the relation was inversed at later phenological stages. The increased nutrient concentrations of the plant mixture, however, cannot with certainty be interpreted in terms of enhanced nutrient availability in the soil as nutrient off-takes in our experiment often did not show the same trend. For example, Ca (in both plant species) and N (in grass) concentrations increased upon N fertilisation but overall concentrations and total off-takes generally decreased. These disparate results were due to a shift in botanical composition of the species mixtures to one dominated by the grass which had lower Ca and N concentrations than the clover, and confirms the decreased N off-take previously found in mixed white clover-perennial ryegrass leys upon N fertilisation (Elgersma *et al*., 2000). The impact of N fertilisation on the botanical composition obviously had a strong impact on the overall nutrient concentrations and total off-takes of the species mixture.

Implications for the feeding value of the species mixture

The tested by-products all affected the botanical composition. Such changes have implications for the feeding value of a species mixture. A balanced diet is of utmost importance for animal productivity and health, and especially so in dairy production. Legumes may increase the concentrations of most macro- and micronutrients in the forage crop as shown here, and by Lindström (2013), Pirhofer-Walzl *et al.* (2011) and Govasmark *et al*. (2005). In addition, legumes have the potential to increase forage digestibility, energy value and the cows´ feed intake, all needed for high milk production (Bertilsson and Murphy, 2003). Where clover-grass leys contribute a large proportion of the diet, ley fertilisation should thus be adjusted so that the desired botanical composition is achieved. The change in overall macro- and micronutrient composition of the mixtures also has implications for the need for mineral nutrient supplementation. In this experiment, biogas digestate and rockdust amendment increased crop mixture concentrations of Cu and Zn to close to or over the minimum concentrations recommended by the US National Research Council (NRC) (2001; Supp. 3a and b). The rockdust and wood ash increased crop Mg concentrations which can be of importance where grass tetany (hypomagnesaemia) occurs.

The data indicate that the by-products can improve both forage quantity and quality on nutrient-poor soils in the absence of fertilisers or as a complement to macronutrient fertilisation. However, the results from pot experiments should always be evaluated cautiously and evaluation via field experiments should be the next essential step. Studies on other aspects of by-product recycling are also likely to be needed to secure safe and long-term sustainable use of by-products as fertilisers. These include more in-depth characterisation of the by-products and a process based understanding of the factors that lead to the observed behaviours. Monitoring of soil quality (e.g. by using soil health indicators and element input-output mass balances of nutrients and non-nutrients) and consideration of long term ecological effects are also needed. The approach to recycle by-products is worth pursuing further, but requires good management skills and knowledge of by-products characteristics and processes in the soil-plant system, including the effects on species competition and thus composition of species mixtures.

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Supporting information

Supporting information 1 All-season average clover concentration of (A) macronutrients (g kg^{-1} DW) and (B) micronutrients (mg kg^{-1} DW).

Supporting information 2 All-season average grass concentration of (A) macronutrients (g kg^{-1} DW) and (B) micronutrients (mg kg^{-1} DW).

Supporting information 3 Overall (A) macronutrient (g kg^{-1} DW) and (B) micronutrient (mg kg^{-1} DW) concentrations of grass-clover mixture.

Supporting information 4 Accumulated off-take of (A) macronutrients (g m^{-2}) and (B) micronutrients (mg m^{-2}) by the grass-clover mixture.

Supporting information 5 Correlations between plant nutrient concentrations and soil pH, plant biomass (g DW) production and soil EDTA extractable concentrations of the same nutrient.

Table 1 Original characteristics of the two soils (Hollsby and Rådde) used in the pot experiment; total and extractable (AL[a] or EDTA) concentrations of nutrients

Soil	Hollsby		Rådde	
Characteristic	Total g kg^{-1}	AL mg kg^{-1}	Total g kg^{-1}	AL mg kg^{-1}
N	1.9	na	2.8	na
P	0.78	13	1.19	51
K	25	23	20.6	53
Ca	9.98	490	11.4	1020
Mg	3.01	12	3.51	55
S	0.33	na	0.48	na
	Total mg kg^{-1}	EDTA mg kg^{-1}	Total mg kg^{-1}	EDTA mg kg^{-1}
Co	2.6	0.07	3.8	0.02
Cu	6.9	2.3	6.5	0.7
Mn	531	35	431	8
Mo	0.40	bd[b]	0.85	bd
Zn	46	4.6	30	2.3

[a] ammonium lactate/acetic acid solution

[b] below detection limit

Table 2 Original characteristics of the two soils (Hollsby and Rådde) used in the pot experiment. Clay, silt and sand fractions are given as % of the mineral fraction

	Soil	
Characteristic	Hollsby	Rådde
Clay (%)	4	8
Silt (%)	69	31
Sand (%)	27	61
pH_{CaCl2}	4.8	5.2
CEC (cmol+c kg^{-1})	9	13
BS (%)	40	52
SOC (%)	2.2	3.5
Quartz (%)	53	52
K-feldspar (%)	17	15
Plagioclase (%)	19	19
Amphibole (%)	2	4
Dioctahedral phyllosilicates(%)	4	4
Trioctahedra phyllosilicates(%)	3	4
Iron oxides (%)	1	1

Table 3 Characteristics of the waste products biogas digestate (BD), pot ale (PA), rock dust (RD) and wood ash (WA), waste product and nutrient application rates used in the experiment. The nutrient or non-nutrient element limiting the application rate of waste products is given in **bold**. Application rates of N, P and K presented separately up to and after second harvest. [a] denotes the limiting element according to SEPA if N application via waste products had not been targeted to 15 g m^{-2}. [b] denotes the nutrient was added in solution. [c] denotes the nutrient was added as dry salt

	Amendment characteristics					Application rates							
	unit	PA	BD	RD	WA	unit	FF	PA	BD	RD-N	RD+N	WA-N	WA+N
Amount product		na	na	na	na	kg m^{-2}	na	8.5	1.5	5.0	5.0	0.14	0.14
Liming effect	% CaO	-4	6.2	1.9	51.3		na	na	na	na	na	na	na
C	%	47	42	0.005	1.1	g m^{-2}	-	126	177	-	-	1.5	1.5
N	g kg^{-1}	**58**	**24**	1.0	0.1	g m^{-2}	13[c] + 2[b]	15+ 2[b]	15+ 2[b]	-	13 + 2[b]	0.008	13 + 2[b]
P	g kg^{-1}	15	8.2	1.2	21	g m^{-2}	18.8+7.5[c]	3.9+7.5[c]	3.4+7.5[c]	6.0+7.5[c]	6.0+7.5[c]	3.1+7.5[c]	3.1+7.5[c]
K	g kg^{-1}	32	12	2.6	69	g m^{-2}	26.5[c]+4.2[b]	8.6+4.2[b]	4.9+4.2[b]	12.8+4.2[b]	12.8+4.2[b]	10.0+4.2[b]	10.0+4.2[b]
Ca	g kg^{-1}	1.7	49	13	324	g m^{-2}	143[c]	0.44	20.3	65.8	65.8	46.9	46.9
Mg	g kg^{-1}	6.0	6.2	17	40	g m^{-2}	19.6[b]	1.58	2.58	84.1	84.1	5.79	5.79
S	g kg^{-1}	4.2	3.1	0.09	0.82	g m^{-2}	3.91[b]	1.10	1.28	0.46	0.46	0.12	0.12
Co	mg kg^{-1}	0.07	0.89	12	21	mg m^{-2}	-	0.02	0.4	59	59	3.0	3.0
Cu	mg kg^{-1}	177[a]	29	7.3	118	mg m^{-2}	4.3[b]	47	12	36	36	17	17
Mn	mg kg^{-1}	16	215	375	7810	mg m^{-2}	98[b]	4.3	90	1850	1850	1130	1130
Mo	mg kg^{-1}	0.45	1.9	0.20	<6	mg m^{-2}	0.04[b]	0.1	0.8	1.0	1.0	0.4	0.4
Zn	mg kg^{-1}	21	76	46	182	mg m^{-2}	0.2[b]	5.6	32	228	228	26	26

Table 4 Soil pH, electrical conductivity (EC) and EDTA extractable nutrient concentrations (macronutrients in g kg^{-1} DW soil; micronutrients in mg kg^{-1} DW) 14 months after fertilisation and by-product amendment; fully fertilized (FF), biogas digestate (BD), pot ale (PA), rock dust (RD) and wood ash (WA). Data given as LSMeans±SEM of four replicates, df = 7

	Control	FF	PA	BD	RD - N	RD + N	WA - N	WA + N
Hollsby								
pH	5.0±0.0	5.1±0.1	4.9±0.1	5.0±0.0	5.0±0.0	5.1±0.1	5.3±0.0	5.4±0.0
EC	93±6	211±16	97±6	104±4	104±4	103±1	118±3	122±3
P	0.026±0.001	0.027±0.002	0.029±0.001	0.029±0.001	0.027±0.002	0.025±0.002	0.027±0.001	0.028±0.001
K	0.020±0.001	0.030±0.002	0.033±0.003	0.027±0.001	0.021±0.001	0.026±0.001	0.034±0.002	0.042±0.001
Ca	0.99±0.03	0.77±0.02	1.03±0.02	1.08±0.06	1.00±0.01	1.04±0.02	1.21±0.02	1.26±0.03
Mg	0.016±0.001	0.040±0.001	0.021±0.001	0.016±0.001	0.022±0.003	0.026±0.001	0.029±0.001	0.033±0.001
Co	0.15±0.01	0.21±0.02	0.13±0.01	0.13±0.01	0.13±0.01	0.13±0.01	0.11±0.01	0.11±0.01
Cu	1.4±0.1	1.1±0.0	2.0±0.1	1.5±0.0	1.4±0.1	1.4±0.1	1.6±0.1	1.6±0.1
Mn	26±1	64±4	22±2	23±1	21±2	22±3	22±2	22±2
Mo	0.025±0.007	0.056±0.027	0.024±0.011	0.018±0.000	0.021±0.003	0.011±0.004	0.019±0.002	0.019±0.002
Zn	3.6±0.2	5.4±0.2	3.7±0.1	3.8±0.3	3.4±0.1	3.2±0.1	4.0±0.5	3.6±0.2
Rådde								
pH	4.6±0.1)	5.0±0.1	4.7±0.1	4.7±0.0	4.7±0.1	4.8±0.1	5.2±0.0	5.2±0.0
EC	85±8	223±8	88±6	100±9	80±2	84±4	112±2	104±3
P	0.010±0.000	0.030±0.004	0.015±0.001	0.014±0.002	0.011±0.001	0.010±0.001	0.014±0.001	0.013±0.001
K	0.015±0.001	0.029±0.003	0.023±0.002	0.020±0.001	0.013±0.001	0.014±0.001	0.028±0.003	0.034±0.004
Ca	0.55±0.01	0.89±0.07	0.56±0.02	0.65±0.04	0.65±0.05	0.56±0.01	0.81±0.01	0.82±0.01
Mg	0.005±0.000	0.040±0.002	0.009±0.001	0.007±0.000	0.014±0.000	0.013±0.000	0.018±0.001	0.019±0.001
Co	0.23±0.02	0.21±0.01	0.26±0.01	0.22±0.02	0.22±0.02	0.23±0.01	0.18±0.01	0.19±0.01

Cu	1.1±0.0	1.1±0.0	1.5±0.0	1.2±0.0	1.1±0.0	1.1±0.0	1.2±0.0	1.3±0.0
Mn	73±4	62±3	86±3	72±4	67±4	69±4	60±3	60±5
Mo	0.023±0.004	0.050±0.022	0.019±0.002	0.023±0.005	0.019±0.001	0.021±0.002	0.020±0.003	0.017±0.001
Zn	5.7±0.3	4.9±0.1	5.7±0.3	5.4±0.4	5.9±1.0	5.2±0.2	4.4±0.2	4.2±0.1

Figure captions

Figure 1 Biomass production (g DW m^{-2}) and botanical composition (proportion of clover) in the grass-clover mixture grown on (A) Hollsby and (B) Rådde soil. Data are given as LSMeans with error bars indicating SEM of four replicates, df = 7.

Figure 2 Relative all-season average nutrient concentrations of clover grown on (A) Hollsby and (B) Rådde soil, expressed as a percentage of the unamended control. Data given as LSMeans with error bars indicating SEM of four replicates, df = 6.

Figure 3 Relative all-season average nutrient concentrations of grass grown on (A) Hollsby and (B) Rådde soil, expressed as a percentage of the unamended control. Data given as LSMeans with error bars indicating SEM of four replicates, df = 6.

Figure 4 Relative overall nutrient concentrations of grass-clover mixture grown on (A) Hollsby and (B) Rådde soil, expressed as a percentage of the unamended control. Data given as LSMeans with error bars indicating SEM of four replicates, df = 6.

Figure 1A

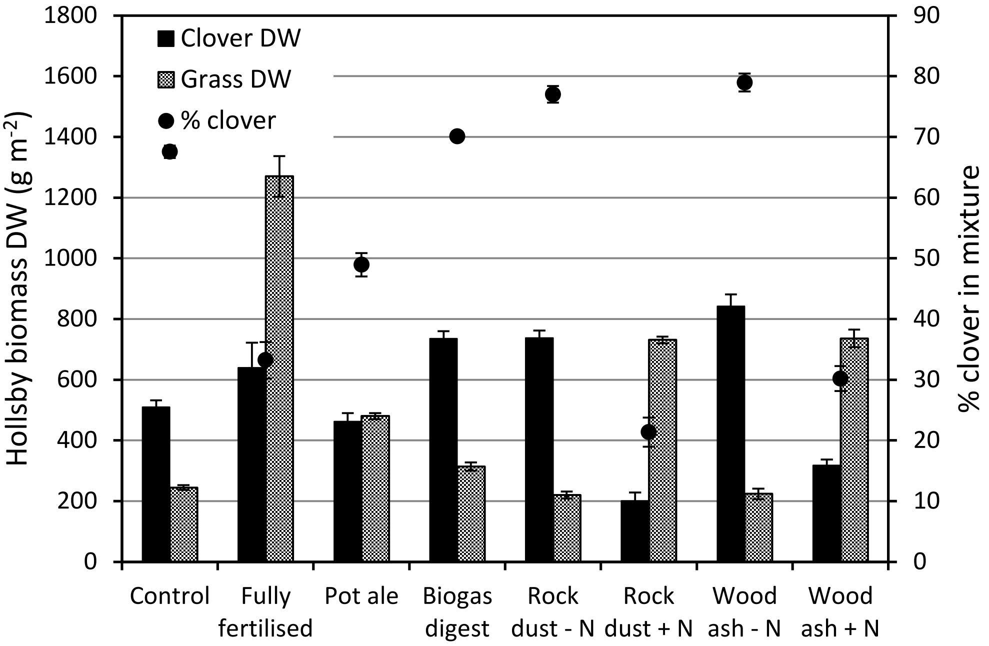

Clover DW
Grass DW
% clover
Hollsby biomass DW (g m-2)
% clover in mixture
1800
1600
1400
1200
1000
800
600
400
200
0
90
80
70
60
50
40
30
20
10
0
Control
Fully fertilised
Pot ale
Biogas digest
Rock dust - N
Rock dust + N
Wood ash - N
Wood ash + N

Figure 1B

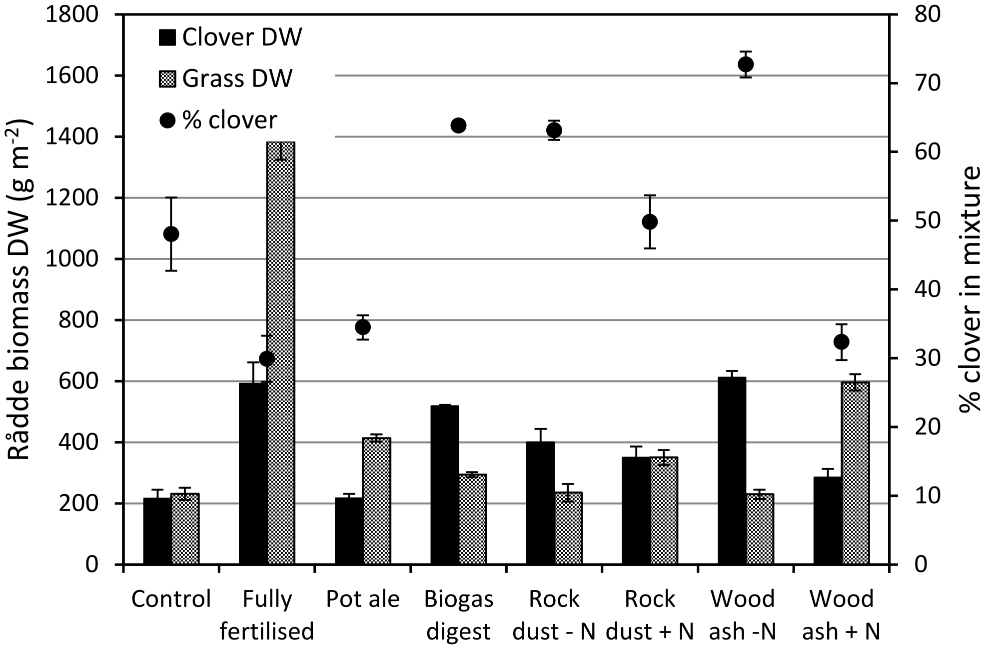

Rådde biomass DW (g m-2)
% clover in mixture
■ Clover DW
▨ Grass DW
● % clover
0
200
400
600
800
1000
1200
1400
1600
1800
0
10
20
30
40
50
60
70
80
Control
Fully fertilised
Pot ale
Biogas digest
Rock dust - N
Rock dust + N
Wood ash -N
Wood ash + N

Figure 2A

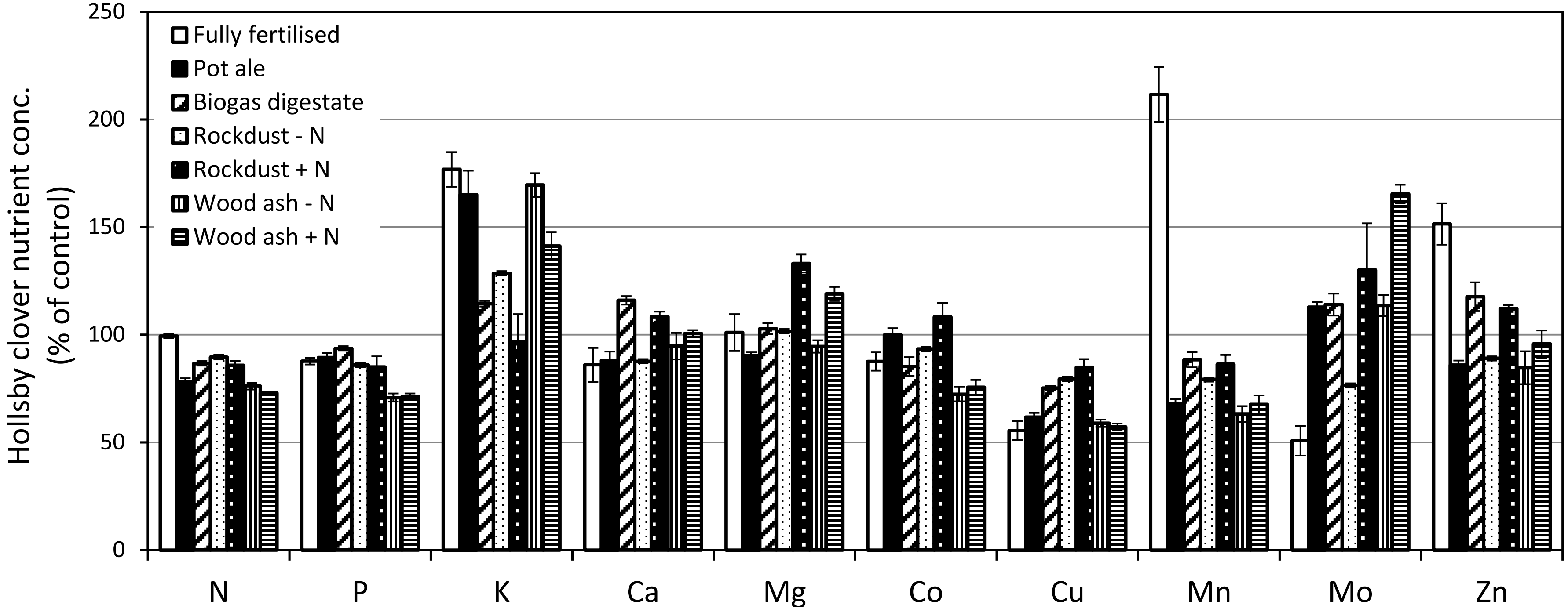

Hollsby clover nutrient conc. (% of control)
0
50
100
150
200
250
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn

Figure 2B

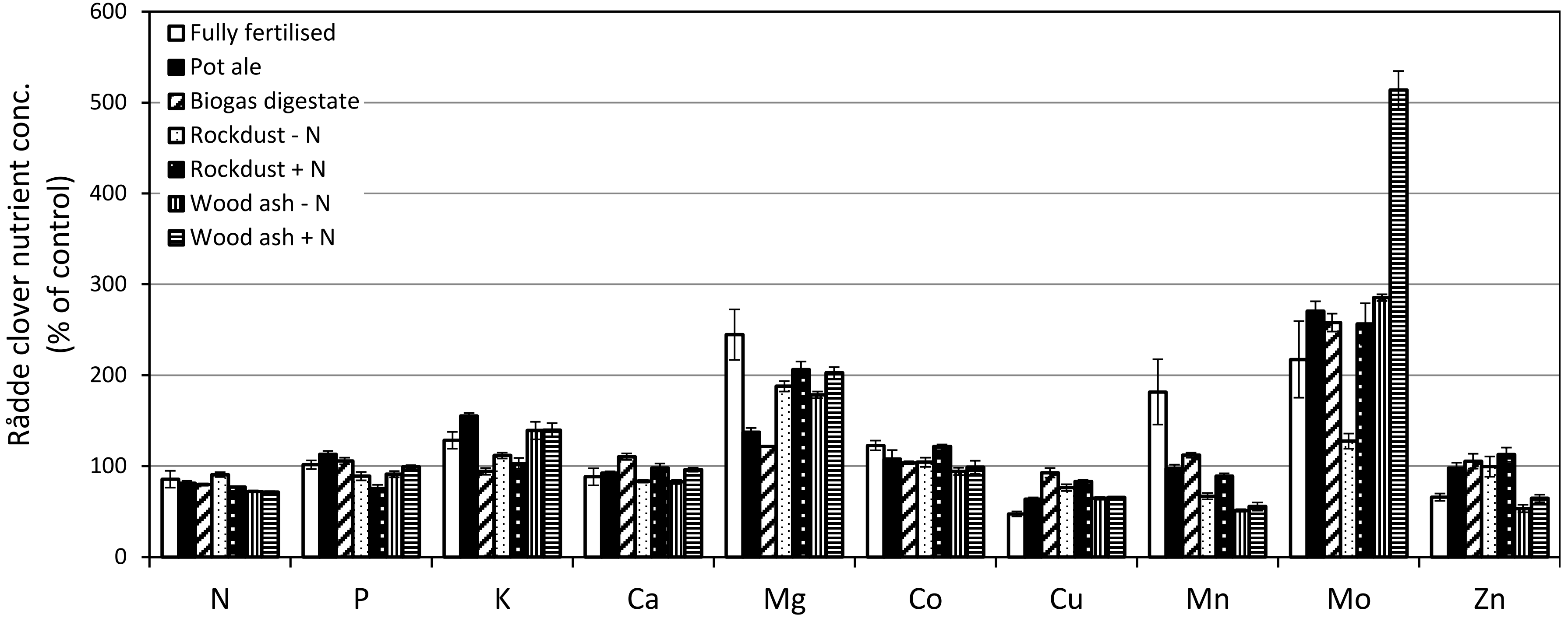

Rådde clover nutrient conc. (% of control)
600
500
400
300
200
100
0
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn

Figure 3A

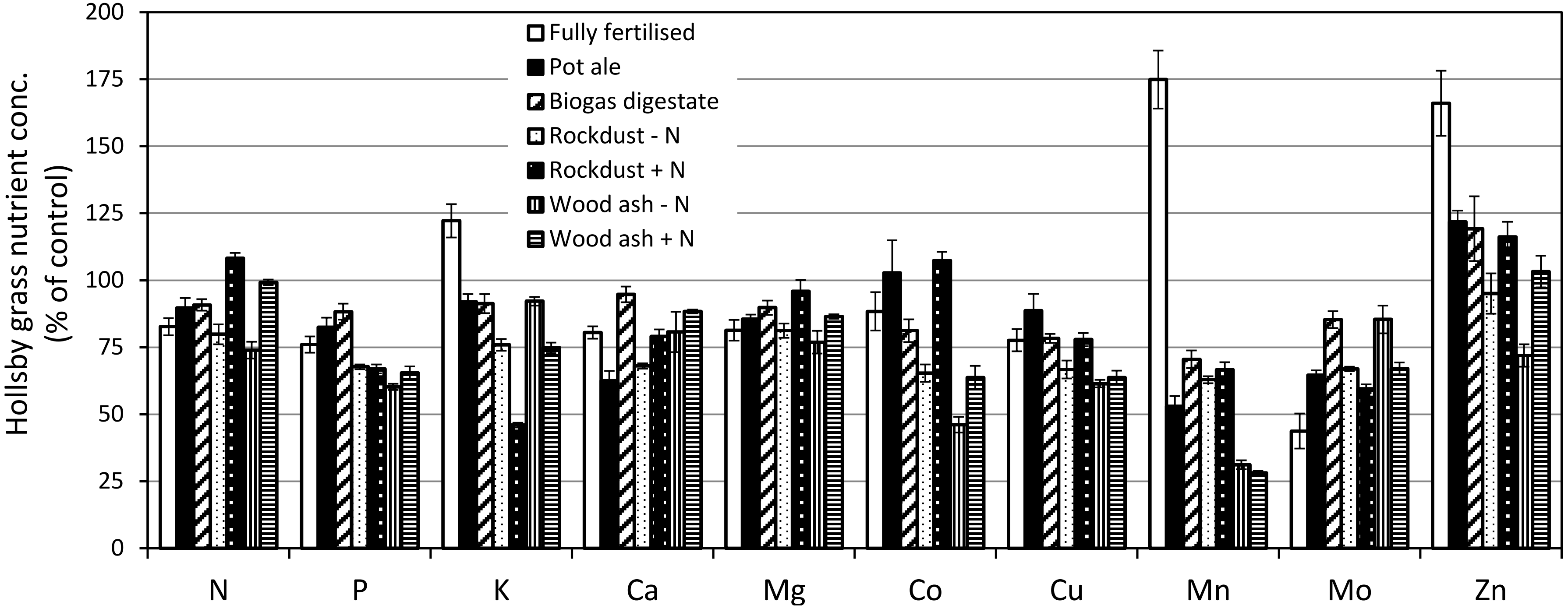

Hollsby grass nutrient conc. (% of control)
200
175
150
125
100
75
50
25
0
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn

Figure 3B

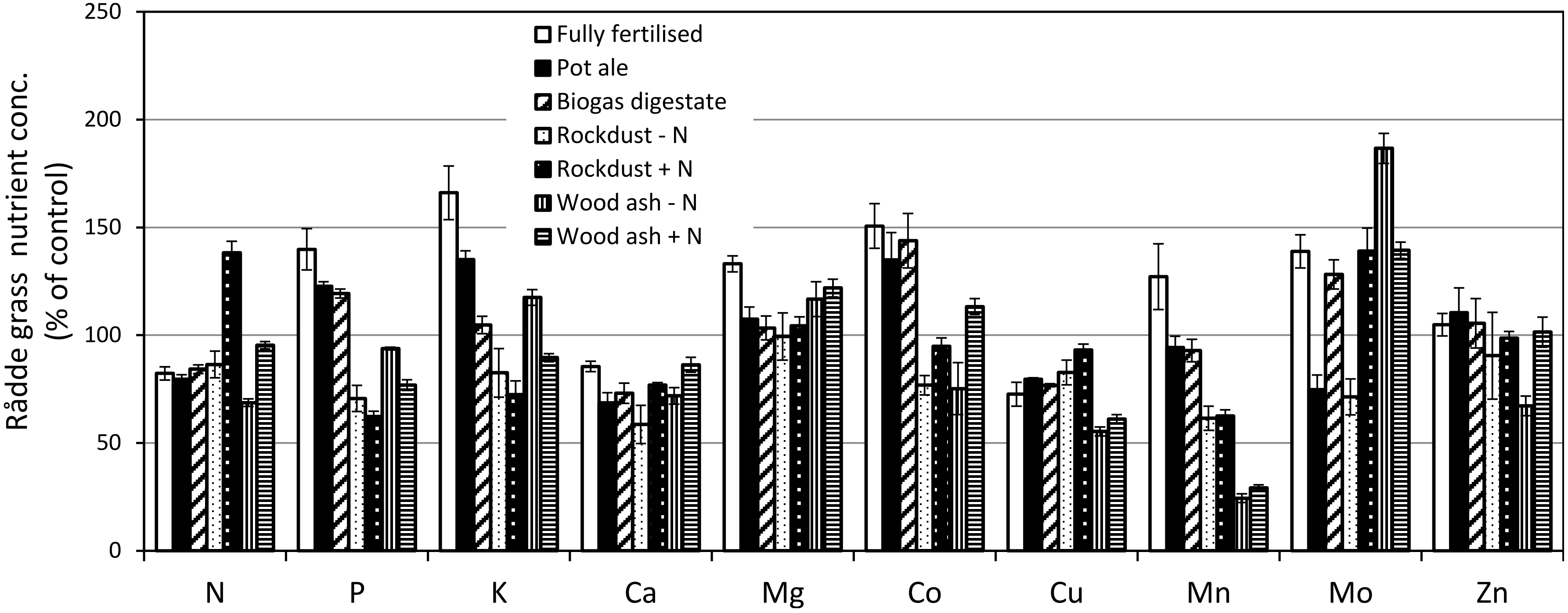

Rådde grass nutrient conc. (% of control)
250
200
150
100
50
0
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn

Figure 4A

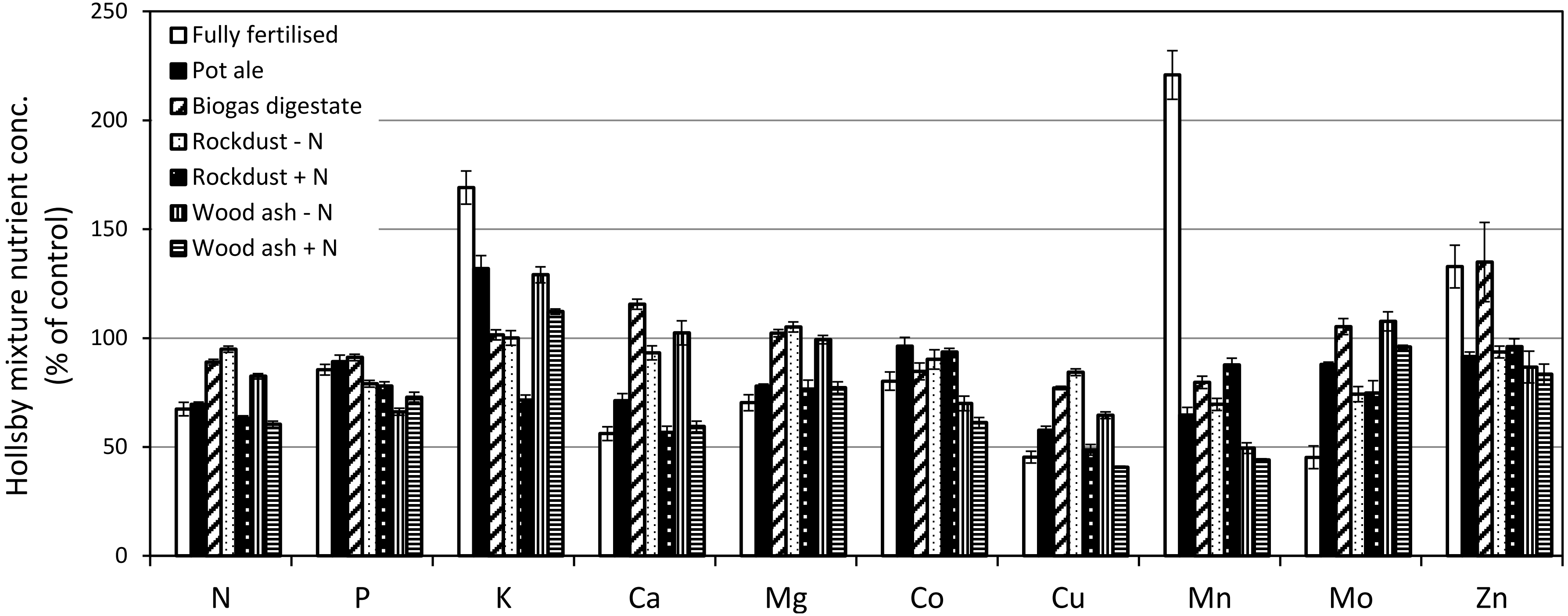
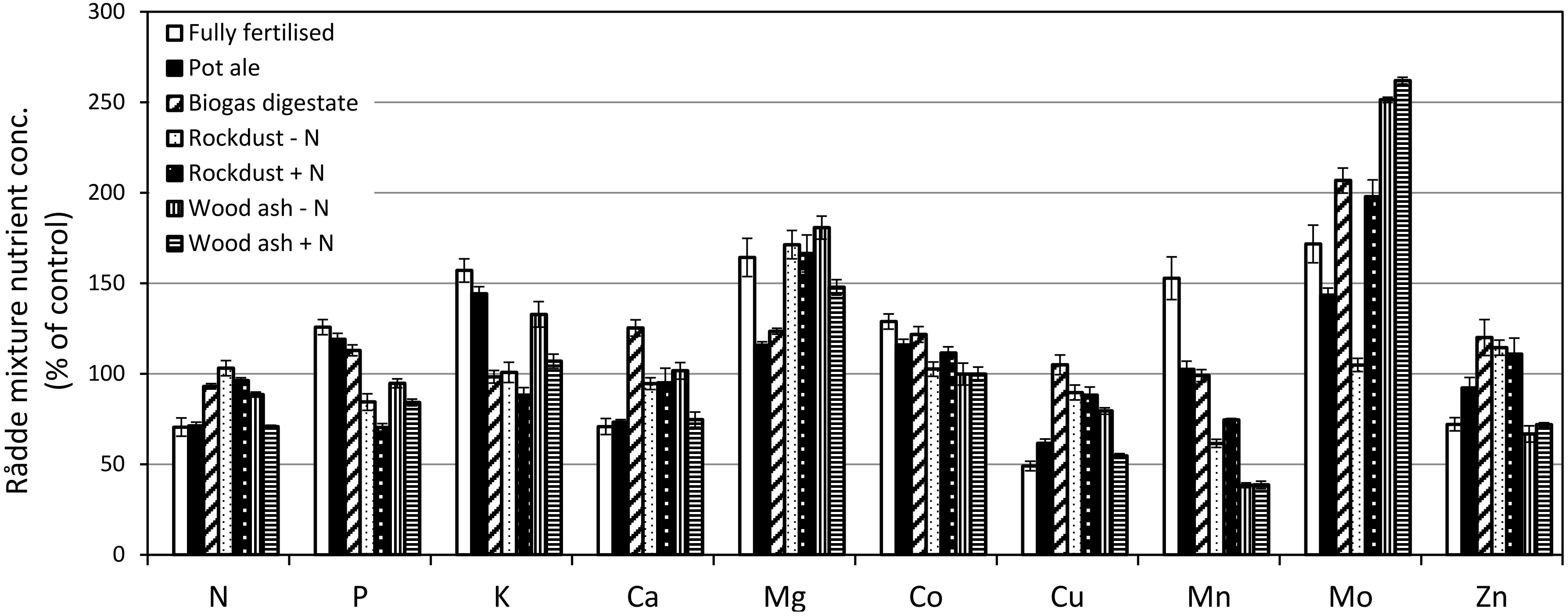

Hollsby mixture nutrient conc. (% of control)
250
200
150
100
50
0
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn

Figure 4B


Rådde mixture nutrient conc. (% of control)
300
250
200
150
100
50
0
Fully fertilised
Pot ale
Biogas digestate
Rockdust - N
Rockdust + N
Wood ash - N
Wood ash + N
N
P
K
Ca
Mg
Co
Cu
Mn
Mo
Zn