Boron fertilisation of organically managed grass-clover swards on coarse-textured soils: effects on botanical and element composition

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Three trials were performed on two organic farms with dairy and suckler cows and using home-produced forage and feed crops, predominantly grass-clover ley, in order to determine whether boron (B) is a limiting factor for legumes on coarse-textured soils in an area predisposed to low B soil concentrations. The effects of B fertilisation (applied as sprayed liquid) on biomass yield, botanical composition and plant macro- and micronutrient concentrations relative to soil concentrations and livestock requirements were investigated. Boron fertilisation (i) did not affect any yield, (ii) increased the white clover percentage significantly in forage on one farm and (iii) increased B concentrations in plants and soil on both farms, and (iv) did not affect concentrations of other nutrients in forage on either farm. Thus, B was not an obvious limiting factor on these farms. Effects of management practices on interactions and ratios between B, calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) and their implications are discussed.

Key words: deficiency, interaction between nutrients, legumes, livestock requirement, macronutrient, micronutrient

Introduction

Organic cropping systems mostly rely on site-specific conditions such as soil mineral composition, ecosystem services from soil-borne organisms and crop rotations supporting soil structure, soil fertility pest and weed control (IFOAM 2005). In low input production systems, there may potentially be inadequate concentrations of essential micronutrients for plants, animals and humans due to poor parent material, that have to be considered (Watson et al. 2012). In organic certified production, mineral fertilizers are not generally allowed, according to the rules (KRAV 2015), but there are exceptions. Micronutrient application with inorganic fertilizers in field crops is permitted under circumstances with a confirmed deficiency situation. Rotations with a mixture of legume crops, e.g. grass/clover leys, providing nitrogen (N) through dinitrogen (N_2) fixation and crops making use of N and other rotational advantages are crucial for a functioning system (Weller and Bowling 2007). Effective N_2 fixation requires essential minerals in balanced proportions to optimise the legume/rhizobia symbiosis (Bonilla and Bolaños 2009). A successful establishment of legumes in ley crops is a prerequisite for the productivity of the system.

In four conference publications (Plant and Soil, the whole volume 1997:193, Randall et al. 1991, Goldbach et al. 2002, Xu et al. 2007), a corporation of a multidisciplinary team, collected and updated knowledge about plant, animal and human boron (B) nutrition. Relying on these publications, a review from an agricultural and food systems perspective was carried out (Linse et al. 2011).

The Nordic region is one of five major areas in the world in which B deficiency can be expected in crop production (Shorrocks 1997). The Scandinavian granitic and gneissic rocks in particular have low B concentrations (on average 4 mg kg^{-1}), compared with 10 mg kg^{-1} in European granites (Wikner 1983). However, cropping history, management regimes and local climate conditions are also important for actual soil conditions and in that perspective, soil mapping is only one factor among many (Shorrocks 1997, Watson et al. 2012).

Boron is essential for plants and has e.g. a central role in stabilising cell walls and cell membranes (Bonilla and Bolaños 2009). It is especially important where new tissues are created, such as growing points and flower production and, in legumes, also in all steps of the symbiosis with N_2-fixing rhizobia. Calcium (Ca) interacts with B in these physiological processes and the soil Ca:B ratio is important. Boron is also reported to be beneficial for mycorrhizal root colonisation, thereby improving the nutrient balance in host plants (Lambert et al. 1980). A *permanent risk* of B deficiency occurs: (i) in areas with the parent material low in B, (ii) when soil solution

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pH > 6.3-6.5, (iii) in coarse-textured soils, (iv) when the proportion of organic material (OM) in soil is low and (v) in humid climates. *Temporary risks* of B deficiency arise due to e.g. (i) drought periods, (ii) high growth rates caused by macro-nutrient sufficiency and suitable environmental factors, (iii) large harvests of green fodder or heavy grazing, and (iv) liming of acid soils (Gupta 1993b, Shorrocks 1997).

Boron interacts with the cations K^+, Ca^{2+}, Mg^{2+} and Na^+ in soils and plants (Tariq and Mott 2006). The type and amount of liming material, the target pH and risks of antagonism between ions of K^+, Ca^{2+} and Mg^{2+} have to be taken into account before liming, since the ratios between these elements are more important than their individual concentrations (Loide 2004).

Determining the concentrations of macro- and micronutrients and comparing these against recommended levels is one way to evaluate the nutrient quality of forage (Suttle 2010), but recommendations for animal requirements differ between countries and are not absolute values. Every situation is unique, depending on animal breed, age and production intensity, together with environmental conditions (Suttle 2010). Sward species composition (grasses, legumes and herbs) also influences the nutrient composition of forage, as element concentrations vary between species (Pirhofer-Walzl et al. 2011, Lindström et al. 2013). Even weeds can be of interest in this context (Johansen 2009).

Boron is classified as an occasionally beneficial element for higher animals and humans, but it does not meet all criteria to qualify as an essential element, e.g. since no biochemical function in higher animals and humans has been defined (Suttle 2010, Nielsen 2014a). As deficiency and toxicity situations of these elements can cause severe chronical diseases, Nielsen (2014a) emphasizes that dietary intake guidance should be given. Nielsen (2014b) summarizes beneficial effects of nutritional amounts of B, on e.g. arthritis, bone growth and maintenance and the central nervous system, for humans and higher animals fed low B diets.

The overall aim of the present study was to evaluate the influence of B fertilisation in organically managed mixed grass-clover swards and to determine whether B is a limiting factor for legumes in areas where soils are geologically predisposed to low B contents. Specific objectives were to investigate the effect of B fertilisation on: (i) biomass yield, (ii) botanical composition, (iii) B concentration in soils and plants and (iv) interactions between B and other nutrients. The hypotheses were that B fertilisation on low B soils (i) increases yield, (ii) increases the clover content in the sward, (iii) increases B concentrations in plants and soil and (iv) affects plant concentrations of other nutrients.

For this purpose, trials were carried out on two organic livestock farms with dairy and suckler cows based on home-produced perennial forages and annual crops. The effect of different management practices, such as slurry fertilisation and liming, on the specific objectives was considered. The concentrations of macronutrients and micronutrients in the forage mixtures were compared against the requirements of lactating and dry dairy cows.

Materials and methods

Study sites

A B fertilisation study was carried out in 2009 in south-west Sweden, in an area with a humid climate and with geological conditions (gneissic and granite parent material) giving low B concentrations in soil (Eriksson et al. 1997). Thirty-year average (1961–1990) precipitation at two nearby meteorological stations, Borås and Ulricehamn, is 955 and 908 mm year^{-1}, respectively, and mean temperature in the seven months April-October is 4, 10, 14, 15, 14, 10 and 6 °C (SMHI). The soils in the region are coarse-textured and prone to leaching. The main farming activities are ruminant, livestock production and fodder crops such as mixed grass/clover leys for forage and grazing. Problems with low soil K availability in ley cropping have been identified (Salomon 1999, Jansson 2008). With increasing ley age, the yield, clover proportion and concentrations of plant K and even Mg have a tendency to decrease (Jansson 2008).

Trials were developed in two locations and specific details will be provided for each site and soil type. Three identical trials were established in first-year mixed grass/clover leys. Two of these trials were carried out in a field on the commercial farm Borrarp (57°37,370´N, 13°31,529´E) and one trial was performed at Rådde research station, The Rural Economy and Agricultural Society Sjuhärad (57°36,368´N, 13°15,676´E). Both fields are organically managed and certified (KRAV), for 20 and 15 years, respectively, at the time of the trials (summer 2009). The trials started after the first cut (Borrarp 4 June, Rådde 8 June). The B treatments were applied on 29 June and 3 July, respectively, and the study was terminated after the second cut (27 July and 5 August, respectively).

Borrarp: The Borrarp soil is a loamy sandy till, with about 8% OM, pH 6.2, total B (B_{HNO3}) 1.9–2.2 mg kg^{-1} and plant-available B (B_{hws}) 0.39–0.46 mg kg^{-1}. In 2008, prior to spring sowing, the whole field was fertilised with 30 ton ha^{-1} of deep litter cattle manure and limed with 2 ton ha^{-1} dolomite ($CaMg[CO_3]_2$). In 2009, one trial was fertilised with 20 ton ha^{-1} cattle slurry after the first cut (Borrarp+S), while the other trial received no slurry (Borrarp-S). The previous crop sequence consisted of: 2005-2007 grass/clover ley, 2008 barley (*Hordeum vulgare* L.)/pea (*Pisum sativum* L.), under-sown with grass/clover mixture (SW381; 14% red clover (*Trifolium pratense* L. cvs. SW Ares and Sara), 10% white clover (*Trifolium repens* L. cvs. Ramona and Sonja), 27% timothy (*Phleum pratense* L. cv. Ragnar), 24% meadow fescue (*Festuca pratensis* L. cv. Sigmund) and 25% perennial ryegrass (*Lolium perenne* L. cv. Helmer).

Rådde: The Rådde soil is a sandy loam till with 6% OM, pH 6.2, (B_{HNO3}) 1.7 mg kg^{-1} and B_{hws} 0.3 mg kg^{-1}. The previous crop sequence was: 2004-2006 grass/clover ley, 2007 barley, 2008 sparse barley under-sown with grass/clover mixture (SW 348; 10% red clover (cvs. Sara and SW Ares), 5% white clover (cv Ramona), 55% timothy (cv Ragnar), 20% meadow fescue (cv Kasper) and 10% perennial ryegrass (cv Helmer). In 2008 before spring cultivation, deep litter bedding from cattle (20 ton ha^{-1}) but no lime was applied to the field. No cattle slurry was added in 2009 (Rådde).

Study design and implementation

Each trial had a randomised complete block design with two treatments, 0 g B ha^{-1} (-B) and 450 g B ha^{-1} (+B), in four replicates (blocks), and plot size 3.2 m × 10 m**.** A liquid fertilizer was used, since the study was conducted in already established ley crops on farms with certified organic production. The authors applied for an exception from the rules about the requirement of a documented boron deficiency situation. The application was approved. According to the recommendations by Rio Tinto minerals (Greenwood Village, CO, USA), 3 L "Bortrack 150" in 200 L water ha^{-1} was sprayed on plants and open soil surfaces after the first cut when the crop covered 50% of the soil surface. The B compound in the fertilizer "Bortrack 150" was borethanolamin (a complexation between boric-acid and monoethanolamin) diluted in water to 150 g water soluble B L^{-1} and with surfactants added. Time and environmental conditions for the treatment: Borrarp+, Borrarp- (2009-06-29, 2200h, no wind, 90-100% RH, 15–20°C, daytime warm and dry, heavy rainfall in the afternoon the day after) and Rådde (2009-07-03, 0730 h, no wind, 64% RH, 20°C).

In conjunction with B fertilisation, the botanical composition (respective percentage) of (i) red and white clover (total clover), (ii) sown grass species and grass weeds (grass) and (iii) dicotyledonous weeds (dicot weeds) was visually determined to be: Borrarp+S 20, 75 and 5%; Borrarp-S 19, 76 and 5%; and Rådde 60, 40 and <1%.

Sampling and analyses

Borrarp: Biomass was harvested by hand from 4 x 0.25 m^2 squares randomly placed across the plot, using stainless steel garden scissors. The plants were cut 0.07 m above soil level and pooled into clean non-woven textile bags for protection against mechanical injury and soil contamination. A random subsample for detailed examination was taken and the remaining plants (main sample), intended for determination of dry matter (DM) concentration and yield (kg DM ha^{-1}) were chopped and placed in perforated plastic bags (for further sample preparation, see below). Soil samples were taken for chemical analyses from the topsoil (0–20 cm) in the harvested 4 x 0.25 m^2 squares, with three cores per square, i.e. 12 random cores per plot, and pooled into one sample.

Rådde: Before harvest, 12 paired plant/soil samples randomly distributed across each plot were taken for detailed examination. The plant material from each plot was pooled in textile bags. For the soil sample, one core from the topsoil (0–20 cm) was sampled at every spot where plant material was sampled. A plot harvester (Haldrup, grass harvestor, Løgstør, Denmark) was subsequently used to harvest 15 m^2 of the plots, with a stubble height of approximately 0.07 m. The harvestor automatically selected and chopped a representative sample (main sample) for DM and yield (kg DM ha^{-1}) determination.

The plants in the subsamples from all three trials were prepared for botanical and chemical analyses by separation into grass, red clover, white clover and dicot weeds (referred to as plant types). At Rådde the grasses were mostly sown species and the share of dicot weeds was less than 1% and not further examined. Aerial plant parts (comparable to forage harvest) were used for the chemical analyses, since these were intended to determine forage quality in terms of essential and beneficial elements. The fractions were chopped and placed in separate perforated plastic bags. The main samples and plant type fractions were weighed and placed in a hay drier

(20-25 °C) for 3 days and then dried in forced air at 54 °C in a drying cabinet for 24 hours. After cooling to room temperature, DM content was determined. Dry matter yield was calculated from the dry weight of the main sample and the individual plant type fractions. The botanical composition was expressed on a DM basis for the individual plant types.

The dried plant samples were milled using a blender (Grindomix GM 200, Retsch Gmbh, Haan, Germany) with titanium blade and plastic container to avoid contamination (Dahlin et al. 2012). The plant samples were analysed by ICP-AES (NMKL 161 1998 mod) for Ca, K, Mg, Na, phosphorus (P), sulphur (S), B, copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) after wet digestion in 70% nitric acid (HNO_3) in a microwave oven. Total N was analysed according to the Dumas method on LECO (ISO 16634-2:2009).

The soil samples were air-dried, sieved using a 2 mm mesh plastic sieve and placed in plastic jars. Analyses were as follows: (i) pH: soil was shaken in H_2O, 1:5 (SS-ISO 10390:2007), (ii) organic matter (OM), loss of ignition, 500 °C, 4 hours (KLK no. 1 1965 mod), (iii) plant-available fraction of Ca, K, Mg, P (ammonium lactate (AL): 2.5 g soil was shaken in 50 ml AL (Ca_{AL}, K_{AL}, Mg_{AL} and P_{AL}) (Egnér et al. 1960) (SS028310/ T1, ver. 1, SS-EN ISO 11885:2009), (iv) K, P and Cu reserves: 1 g soil was autoclaved in 2 M HCl, 90 min (K_{HCl}, P_{HCl} and Cu_{HCl}) (Egnér et al., 1960) (KLK no. 1, 1965, mod. SS-EN ISO 11885:2009), (v) plant-available B: hot water-soluble (B_{hsw}), 12.5 g soil were boiled for 5 min in 25 ml water (Berger and Trough 1939), modified version SLL method 11, 1979 mod., water extraction SS-EN ISO 11 885:2009) and (vi) B `reserves´: 3 g soil were autoclaved with 20 ml 7 M HNO_3 in a microwave oven (B_{HNO3}). Final determination was made by ICP-AES for analyses (iii)–(vi).

Calcium:B ratio in plants, K and Mg ratio in plants and soil (K:Mg and K_{AL}:Mg_{AL}), Ca and Mg ratio in soil (Ca_{AL}:Mg_{AL}) and correlations between B, Ca, K, Mg and Na in plants and soil were calculated. Forage mixtures: Forage quality in terms of macro- and micronutrients (measured as concentrations of individual elements) was calculated as a weighted mean based on botanical composition and concentrations of elements in the different forage plant types. The Swedish guidelines were used for the evaluation (Spörndly 2003).

Statistical methods

All statistical analyses were carried out using the mixed procedure in SAS System, version 9.3 (Littell et al. 2006). Two models were applied. Model 1 was used for estimating and testing main effects and interactions of trials and treatments on yield, variables measuring botanical composition and soil characteristics (macro- and micronutrient concentrations, pH and OM). This standard model included fixed effects of trials, treatments and treatment-by-trial interaction, and random effects of blocks. The block effects and the residual error effects were assumed to be independent and normally distributed with expected value zero.

Since Model 1 did not allow comparisons between variables, Model 2 was used for this purpose. In this multivariate model, all observations of the original variables, i.e. macro- and micro-nutrient concentrations in plant types and soil, pH, OM, and Ca:B, Ca:Mg and K:Mg ratio, were collected in a single response variable Y. All names of the original variables were collected into a single corresponding explanatory factor, Variable. Model 2 included fixed effects of trials, treatments and variables and all their interactions, and random effects of blocks. The residual error effects were assumed to be independent between plots but correlated (unstructured) within plots. These pairwise intra-plot correlations between the variables were reported and tested using Wald tests and asymptotic standard errors. Not all response variables were included in a single analysis, because the fitting algorithm did not converge, so in practice this analysis was repeated using different subsets of variables that were relevant to compare.

Throughout, the Satterthwaite method was used for estimation of degrees of freedom, and the Tukey method for pair-wise comparisons at significance level 0.05. Since there were no dicot weeds at Rådde, separate analyses were performed for the two trials at Borrarp to evaluate the variables that involved dicot weeds. When needed, observations were log- or square root-transformed before analysis to provide homogeneous variance.

Results

Crop performance and dry matter yield

The +B treatment did not affect forage DM yield (kg ha^{-1}) in any of the three trials (Table 1). The average DM yield (95% confidence interval) was 3359, 1935 and 1647 kg ha^{-1} in Rådde, Borrarp+S and Borrarp-S, respectively. This represented 74 and 104% higher yield at Rådde than at Borrarp+S and Borrarp-S, respectively. The Borrarp+S yield was 18% higher than at Borrarp-S.

Botanical composition – forage mixture

There were no significant trial-by-treatment interactions related to the proportions of the single plant types in the botanical composition of the forage mixtures (Table 1). Averaged over trials, the +B treatment (with near significance, p=0.056) increased the white clover percentage in the forage mixture, compared with the -B treatment. No significant differences were observed for red clover, grass and dicot weed proportions. For white clover there were no significant differences between the trials. Red clover and grass percentages differed between Borrarp and Rådde (Table 1), confirming the visual estimates of sward botanical composition at the start of the study. The proportion of red clover was higher at Rådde than at the two Borrarp trials, but for grass the result was the opposite. Red clover, grass and dicot weed proportions did not differ significantly between the two trials at Borrarp.

Table 1. Biomass yield (DM kg ha^{-1}) and botanical composition (DM basis) of the ley crop. The LSMEANS are given, with the upper and lower values within brackets. Significant differences ($p < 0.05$) between treatments are indicated by different superscripts within columns.

Trial/Treatment	Yield,DM kg ha^{-1}	Red clover, %	White clover[1], %	Grass[1], %	Dicot weeds[3], %
Borrarp+S-B	1920^{b} (1761, 2079)	9^{b} (5, 13)	12^{b} (8, 15)	67^{a} (61, 74)	12 (7, 18)
Borrarp+S+B	1950^{b} (1790, 2109)	7^{b} (3, 11)	13^{ab} (9, 16)	65^{a} (58, 72)	14 (9, 20)
Borrarp-S-B	1631^{b} (1471, 1790)	12^{b} (8, 16)	12^{ab} (9, 16)	65^{a} (58, 72)	11 (6, 17)
Borrarp-S+B	1663^{b} (1504, 1822)	14^{b} (10, 17)	19^{a} (16, 22)	61^{a} (54, 67)	7 (1, 12)
Rådde-B	3334^{a} (3174, 3493)	61^{a} (57, 65)	13^{ab} (10, 16)	25^{b} (19, 32)	-
Rådde+B	3384^{a} (3225, 3543)	57^{a} (53, 61)	13^{ab} (9, 16)	31^{b} (24, 38)	-
p values[2]					
Trial*treatment:	0.987	0.235	0.071	0.300	0.147
Trial:	< 0.001	< 0.001	0.091	< 0.001	0.654
Treatment:	0.538	0.409	0.056	0.883	0.210

[1]Grass includes sown species and grass weeds; [2]Significance $p < 0.05$; [3]Only Borrarp.

Total clover: In terms of total clover proportion, the trial-by-treatment interaction was significant ($p = 0.016$), since at Borrarp+S, the observed difference between the +B treatment and the –B treatment was positive, but at Rådde it was negative. There was no difference in total clover percentage between the two B treatments in any of the three trials. Nevertheless, the total clover percentage in the +B treatment in Borrarp-S was significantly higher than in the two treatments in Borrarp+S (+B $p = 0.011$ and –B $p = 0.010$). Averaged over treatments, the proportion of total clover was higher at Borrarp-S than at Borrarp+S ($p = 0.006$).

Element concentrations in soils and plant tissues

Boron concentrations

Figure 1 gives an overview of effects of B fertilisation on B concentrations in soil, red clover, white clover, grass, dicot weeds and forage mixtures. For soil, there was no significant interaction between trials and treatments in B_{hws} (mg kg^{-1}) ($p = 0.119$). However, the main effects of treatments ($p < 0.001$) and trials ($p = 0.015$) were significant. Averaged over trials, the +B treatment increased the B_{hws}. Averaged over treatments, the $B_{hws,}$ was significant higher at Borrarp+S than at Rådde ($p = 0.013$). The B_{hws} at Borrarp-S did not differ significantly from the B_{hws} in the other trials.

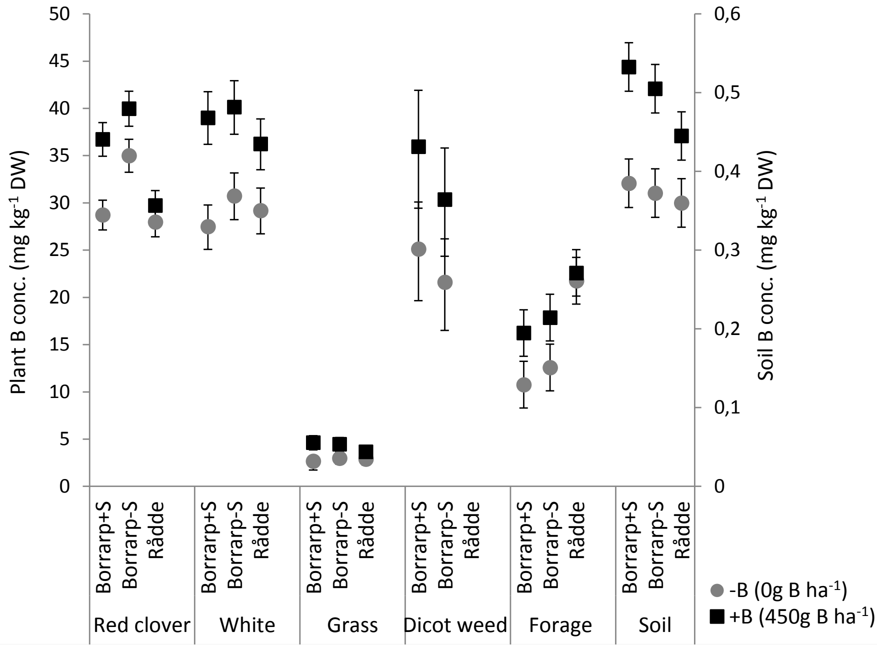

Fig. 1. Effect of boron (B) fertilisation in Borrarp+S (with cattle slurry), Borrarp-S (without cattle slurry) and Rådde on B concentrations in red clover, white clover, grass, dicot weeds, forage mixtures and soil. Error bars indicate 95% confidence intervals.

In the analysis of plant B concentrations, there were significant interactions between (i) trial and treatment (p = 0.022), (ii) plant types and treatment ($p < 0.001$) and (iii) plant types and trial (p < 0.001), but the three-way interaction was not significant ($p = 0.857$). The +B treatment increased B concentrations in white clover in all three trials and in red clover and forage mixtures in the two Borrarp trials. At Rådde, there were no significant differences in B concentration between the treatments in red clover, grass and forage mixtures. With the +B treatment, red clover had higher B concentrations in the two Borrarp trials than in the Rådde trial. However, due to the high red clover percentage, the forage mixtures from both treatments at Rådde had significantly higher B concentrations than the mixtures from the two treatments at Borrarp+S and the –B treatment in Borrarp-S. In grasses, the +B treatment only increased the B concentration in Borrarp+S.

Soil variables and plant concentrations of other nutrients

The B treatment did not affect soil and plant concentrations of any nutrient other than B and, as a consequence, concentrations of these elements are reported as trial variations (Tables 2 and 3).

The soil analysis and fertility classes (five classes; K_{AL} I-V, K_{HCl} 1–5) showed that all three experimental soils were low in plant-available K_{AL} (I–II) and storage K_{HCL} (class 1) (Table 2). Plant-available P_{AL} and storage P_{HCL} were adequate in Borrarp (classes IV and 5) and in Rådde (III and 4). The two soils at Borrarp had higher concentrations of Mg_{AL}, P_{AL}, K_{HCL}, P_{HCL}, Cu_{HCl} and percentage of OM than the Rådde soil. The soil in Borrarp+S had a higher pH and concentration of Ca_{AL} than the Rådde soil.

Table 2. Differences between trials in soil conditions, pH, organic matter (OM), extractable element concentrations (mg kg^{-1}) and Ca_{AL}:Mg_{AL} and K_{AL}:Mg_{AL} ratio in the plots after harvest. LSMEANS with different superscripts within columns are significantly different.

Trial	pH	OM (%)	Ca_{AL} (mg kg^{-1})	K_{AL}	Mg_{AL}	P_{AL}	K_{HCL}	P_{HCL}	Cu_{HCl}	$Ca_{AL}Mg_{AL}$[1]	$K_{AL}Mg_{AL}$[1]
Borrarp+S	6.3^{a}	8.3^{a}	2038^{a}	51^{a}	111^{a}	82^{a}	463^{a}	1049^{a}	6.8^{b}	18^{ab}	0.46^{b}
Borrarp-S	6.3^{ab}	7.4^{b}	1713^{ab}	47^{a}	98^{a}	81^{a}	474^{a}	1100^{a}	8.0^{a}	18^{b}	0.48^{b}
Rådde	6.2^{b}	6.3^{c}	1425^{b}	47^{a}	64^{b}	52^{b}	405^{b}	757^{b}	4.8^{c}	22^{a}	0,74^{a}
p-value	0.021	<0.001	0.048	0.35	<0.001	<0.001	0.008	<0.001	<0.001	0.042	<0.001

[1]Ca_{AL}:Mg_{AL} and K_{AL}:Mg_{AL}: The ratio of soil concentrations of Ca/Mg and K/Mg (important for plant and animal health)

Nutrient concentrations as an effect of plant types over trials differed significantly ($p < 0.001$). Grass had the highest concentrations of K, P, S and Mn, white clover of N, Na and Fe and red clover of Ca, Mg, Cu and Zn. At Borrarp, dicot weeds, dominated by yarrow (*Achillea millefolium*), had the highest concentrations of K, P, Fe and Zn. Nutrient concentrations of forage mixtures also were affected by trial (Table 3). The forage mixtures from Rådde had the highest concentrations of N, K, and Ca and, together with Borrarp-S, also of Cu. Phosphorus and Na concentrations were highest in the mixtures from the two trials in Borrarp, Mn only in the Borrarp-S mixtures and Mg, S and Fe concentrations in the mixtures did not differ between trials.

Table 3. Plant type-by-trial effects of nutrient concentrations in red clover, white clover, grass, dicot weeds and trial effects for forage mixture, related to the requirements of lactating and dry dairy cows. The macro elements (Ca, K, Mg, N, Na, P, S) are given in g kg^{-1} dry weight and the micro elements (Cu, Fe, Mn, Zn) in mg kg^{-1} dry weight.

Plant types by trial	Ca	K	Mg	N	Na	P	S	Cu	Fe	Mn	Zn
	g kg^{-1}							mg kg^{-1}			
Red clover											
Borrarp+S	25^{ab}	15^{f}	6.2^{a}	29^{c}	0.41^{bcd}	2.9^{d}	1.3^{e}	8.5	45^{c}	36^{d}	28^{ab}
Borrarp-S	29^{a}	13^{f}	6.6^{a}	30^{c}	0.40^{bcd}	3.0^{d}	1.4^{e}	11	47^{c}	49^{bc}	31^{a}
Rådde	18^{c}	25^{c}	3.4^{b}	30^{bc}	0.13^{d}	3.0^{d}	1.5^{de}	10	49^{bc}	28^{e}	23^{cd}
White clover											
Borrarp+S	23^{b}	19de	3.4^{b}	34^{a}	2.2^{a}	3.8^{bc}	1.5^{c}	6.7	56^{abc}	42^{c}	21de
Borrarp-S	22^{b}	15^{ef}	3.4^{b}	33^{ab}	2.0^{a}	3.9^{bc}	1.5^{cd}	7.4	62^{ab}	50^{bc}	22de
Rådde	17^{c}	30^{b}	2.5^{c}	36^{a}	0.65^{b}	3.6^{c}	1.9^{c}	7.3	71^{a}	32^{d}	20^{e}
Grass											
Borrarp+S	5.5^{d}	24^{c}	2.3^{cd}	23de	0.48^{bc}	4.0^{b}	2.1^{ab}	5.9	45^{bc}	51^{b}	23^{cd}
Borrarp-S	6.1^{d}	23^{cd}	2.5^{c}	20^{e}	0.50^{bc}	4.0^{b}	2.3^{b}	7.4	55^{abc}	97^{a}	26^{bc}
Rådde	5.6^{d}	37^{a}	2.0^{d}	24^{d}	0.30^{cd}	5.4^{a}	3.0^{a}	8.0	47^{bc}	77^{a}	26^{bc}
p- value	<0.001	0.026	<0.001	0.002	<0.001	<0.001	0.004	0.310	0.268	0.001	0.001
Dicot weeds[1]											
Borrarp+S	13	29	3.3	21	0.41	5.1	1.8	7.7	67	65	38
Borrarp-S	12	25	3.3	20	0.43	4.7	1.7	8.6	71	95	42
Rådde	-	-	-	-	-	-	-	-	-	-	-
Forage mixture[2]											
Borrarp+S	10^{c}	24^{b}	2.9^{a}	24^{b}	0.71^{a}	4.0^{a}	1.9^{a}	6.5^{b}	49^{a}	51^{b}	25^{ab}
Borrarp-S	12^{b}	20^{c}	3.3^{a}	24^{b}	0.73^{a}	3.9^{a}	2.0^{a}	8.3^{a}	58^{a}	84^{a}	27^{a}
Rådde	14^{a}	29^{a}	2.9^{a}	29^{a}	0.26^{b}	3.7^{b}	2.0^{a}	9.2^{a}	51^{a}	42^{c}	24^{b}
p-value[3]	0.001	<0.001	0.059	<0.001	0.008	0.001	0.436	0.002	0.303	<0.001	0.005
Need of:											
Lactating cow[4]	7[5]	10	2.5		2.2	3.5[5]	2.0	11	20	20	45
/Dry cow	5[5]	5.2	1.2		1.0	3.0[5]	2.0	13	13	18	22
Max.conc[6]	20	30	5		-	10	4.0	35	1250	250	250

[a-f]LSMEANS for red clover, white clove and grass with different superscripts within columns differ significantly in plant type-by-trial interaction. [1]At Borrarp, dicot weeds were included in the forage mixtures but at Rådde they were below 1% and thus excluded. [2]Superscripts showing significance are separate for the forage mixtures and omitted for dicot weeds, due to separate analyses. [3]Trial effects. [4]Lactating dairy cow/dry cow, 600 kg, 40/0 kg milk day^{-1}, [5]20/10 kg DM day^{-1}, 140/50 g Ca and 70/30 g P day^{-1}. [6]Maximum concentration in the total diet (Spörndly 2003)

Relations between elements in soil and plants

Across trials, the +B treatment gave lower Ca:B ratio in plant types ($p = 0.04$) and forage mixtures ($p < 0.001$). Effects of trial and plant types ($p = 0.029$) showed that the Ca:B ratio in red clover was higher in Borrarp+S (774) and Borrarp-S (763) than in Rådde (615). In white clover, the ratio differed between Borrarp+S and Rådde. In grass there were no differences between the trials.

Correlations between B, Ca, K, Mg, and Na concentrations in soil, plant species and forage mixtures showed positive correlations for Ca and B in the forage mixtures ($p = 0.030$, $r = 0.70$), and between K_{AL} and B in the forage mixture ($p = 0.021$, $r = 0.69$). In white clover, a negative correlation was observed between B and Na ($p = 0.015$, $r = – 0.77$).

There were positive correlations between Ca_{AL} and Ca in grass ($p = 0.013$, $r = 0.73$), Mg in grass ($p = 0.014$, $r = 0.71$), Ca in forage mixture ($p = 0.016$, $r = 0.70$), and Mg in forage mixture ($p = 0.009$, $r = 0.79$). There were also positive correlations between Ca and Mg in grass ($p = 0.022$, $r = 0.88$), white clover ($p = 0.027$, $r = 0.66$), and forage mixture ($p = 0.009$, $r = 0.82$). There were negative correlations between K and Na ($p = 0.032$, $r = –0.59$) and K and Ca ($p = 0.025$, $r = –0.65$) in white clover and near significant negativ correlation between K and Mg in red clover ($p = 0.056$, r = –0.51).

Discussion

Site specific properties crucial for nutrient supply in organic production

The challenges for sustainable organic production of food and feed crops are to maintain soil fertility, create balance between input and output of nutrients in the cropping system and produce products of high quality under economically satisfactory conditions (Weller and Bowling 2007). In organic farming inherent site specific characteristics such as soil parent material are crucial production factors to take into account in decisions on cropping system and overall farm management (Watson et al. 2012).

In an area with soils predisposed to low B concentrations, field trials were conducted in mixed grass/clover organic ley in order to evaluate the effect of B fertilisation. The results showed that B fertilisation did not affect biomass yield but increased B concentrations in soil and plants. The botanical composition partly changed and the white clover percentage increased in the forage. Concentrations of other nutrients in the plants were not affected by B fertilisation.

Effects of B fertilisation on dry matter yield and soil and plant B concentrations

Poor establishment and persistence of legumes in ley crops are recurring problems with serious implications in cropping systems dependent on symbiotic N_2 fixation. The forages at Rådde were vigorous, with a high percentage of red clover and yield around 400 kg ha^{-1} above the average level for the area (2920 kg ha^{-1} for first-year ley, 2nd cut), while the swards at Borrarp+S and Borrarp-S were weak, with low red clover content and yields of 1000 and 1300 kg ha^{-1}, respectively, below the average for the area (Jansson 2008) (Table 1). In grass-legume crops it is not common to see yield losses due to B deficiency during vegetative growth (Sherrell 1983a). In a greenhouse study, B concentrations for yield response to B fertilisation were determined in red clover (15–18 mg B kg^{-1}) and in white clover (13–16 mg B kg^{-1}) and symptoms of B deficiency were observed (Sherrell 1983a). At Borrarp, similar symptoms, were observed in white clover, where small plants with red-colour spreading from the leaf edges to the middle of the leaves were present. These deficient plants represented a minor share of the samples used for chemical analysis. The B analyses of red and white clover from all three trials showed sufficient B concentrations in the -B plots, i.e. >20 mg kg^{-1} DM (Sherrell 1983a). Grass B concentrations were at the lower limit of the normal range (3 mg kg^{-1} DM) (Sherrell 1983b) (Fig. 1). Reported critical B concentrations are often related to a specific plant part at a defined plant developmental stage (Gupta 1993a). In this study, the whole above-ground plants were harvested for determination of forage element concentrations and the B concentrations may be less comparable with controlled experiments reported in the literature.

The soil B status, at Borrarp and Rådde of B_{HNO3} was 2 mg kg^{-1} (normal variation 2–100 mg kg^{-1}) (Shorrocks 1997) and of B_{hws} was < 0.5 mg kg^{-1}. In studies in different parts of Finland, Sillanpää (1982) found that soils with B_{hws} < 0.3-0.5 mg kg^{-1} was prone to cause B deficiency. The high OM content in our trial soils was a positive factor for the B supply, but otherwise both sites shared several risks for B deficiency such as coarse texture, humid climate and nutrient-demanding harvesting regime (Linse et al. 2011).

Effects of B on botanical composition

In general forage legumes are more sensitive to B deficiency, than grasses (Gupta 1993a). The increase in white clover percentage over trials was in line with expected effect of the + B treatment (Table 1). The application method, spray fertilisation on plants and soil surfaces, in combination with plant morphology, was a probable reason why white clover responded more to the treatment than red clover. White clover with stolons and a shallow root system had a larger plant surface exposed to the fertilizer, than red clover with upright stalk and tap root. In all three trials, B concentration increased more in white clover with the +B treatment, than in red clover. There was no difference in white clover percentage between the trials, which means that the crucial differences in botanical composition between Borrarp and Rådde was in grass and red clover proportions.

The sward in Rådde developed as a normal first year grass-clover ley, dominated by red clover (Jansson 2008), while the weak sward in Borrarp, dominated by grass and dicot weeds, was an effect of bad establishment of the ley before the study started. The slurry fertilisation in Borrarp +S, which also was the action the farmer chose, to compensate for low N, K and clover content, resulted in a maintained low (~20%) total clover proportion, compared with the +B treatment in Borrarp-S where the total clover increased by around 10%. In the -B treatment in Borrarp-S, there were no such differences. Nitrogen supported grasses on the expense of the clovers (Pirhofer-Walzl et al. 2011).

Interactions between B and other elements in plants

Interactions between B, Ca, K, Mg and Na, were studied in sand culture using radish as test plants by adding different amounts of B to an otherwise equal nutrient solution (Tariq and Mott 2006). The authors found that deficiency or excess of B interacted with all the cations and also affected the relationships between these; the plant concentrations of Ca, Mg and Na decreased, while B and K increased, with higher B concentration. This is consistent with our finding of a negative correlation between B and Na in white clover and a positive correlation between K_{AL} and B in forage mixtures. Boron and K interact in cell membranes, and B deficiency can induce K efflux from leaves due to weakened membranes with changed permeability, and both elements can influence the uptake of other nutrients (Cakmak and Römheld 1997).

The field at Borrarp was limed with dolomite before spring sowing in 2008. A short interval between liming and sowing aggravates the risk of B deficiency (Shorrocks 1997). The Ca:B ratio in leaf tissue has been used as an indicator of B deficiency and toxicity, but its usefulness is limited due to interactions with other nutrients (Gupta 1993a). Our results indicated that the ley crops in Borrarp might have been negatively affected by the liming, due to changed ratios of B and other cations (Ca, K, Mg, and Na) in the plants (Tariq and Mott 2006). The higher Ca:B ratios in red clover at Borrarp than at Rådde could be one reason to the low red clover percentage in the Borrarp swards.

The recent liming at Borrarp probably affected the relationship between other nutrient elements which likely affected the botanical composition and in particular the clover component (Loide 2004). In an Estonian study examining interactions and ratios between Ca, K and Mg in e.g. red clover and with different liming materials, a K_{AL}:Mg_{AL} ratio < 0.6-0.7 decreased yield. Also The Swedish Board of Agriculture (Albertsson 2014) recommended K fertilisation even in high K classes (IV) when the K_{AL}:Mg_{AL} ratio is < 0.7. At Borrarp, the K_{AL}:Mg_{AL} ratio was low (0.4–0.5), while at Rådde it was in the optimal range (0.7–0.8). K_{AL} was at the same low level at both sites (Table 2), but at Borrarp, soil ratios altered by dolomite application accentuated the low K_{AL} values, and all the plant types at Borrarp showed lower K concentrations than those at Rådde. The negative correlation between K and Na and between K and Ca in white clover, indicated that white clover at Borrarp, had replaced K with Na in particular, but also with Ca (Table 3). A near significant negative correlation between K and Mg in red clover indicated that red clover had substituted K with Mg. At Borrarp, red clover lost the competition to the other plant types (Table 3), which resulted in low (deficient) K concentrations, excess Mg and an extremely low K:Mg ratio, which could be another reason for the low red clover proportion in the ley, as excess of Mg can cause root problems (Loide 2004).

Coarse-textured soils are sensitive to deficiencies, but are also predisposed to leaching, luxury consumption and toxicity problems (Shorrocks 1997, Salomon 1999). On these soils application of fertilizer and other soil amendments including liming should be handled with care, and more frequent applications at lower rates are preferable, to avoid above mentioned problems.

Nutrient composition of forage mixtures

The botanical composition of a sward is crucial for the nutrient status of the forage (Pirhofer-Walzl et al. 2011, Lindström et al. 2013). Forage based on legume crops, normally has higher mineral and crude protein content but lower structural fibre content and higher digestion rate of the fibre, resulting in higher intake compared to grasses (Frame et al. 1998, Lindström et al. 2013).

This is true even for B. In the present study (Fig. 1), red and white clover had about 10-fold higher B concentration than the grass fraction. According to Suttle 2010, B deficiency can occur when ruminants are fed grain-based diets. Nielsen (2014a, 2014b) stresses the beneficial health effects of B and the importance of recommendations for adequate diets to avoid chronic diseases, e.g. bone.

Since B is classified as an occasionally beneficial element for animals and humans (Suttle 2010, Nielsen 2014a, 2014b), there are at present no guidelines for feed and food concentrations to ensure adequate intake. As a consequence, in the Swedish guidelines for ruminants (Spörndly 2003), only upper concentration limits are presented for B in the total diet and in drinking water (100 mg B kg^{-1} DM and 5 mg l^{-1}, respectively), and B deficiency is not taken into account as a probable reason, for health problems in the herd. In the forage mixtures from Borrarp and Rådde, B concentrations varied between trials and treatments, with the lowest value in the -B treatment (8 mg kg^{-1}) at Borrarp+S and the highest in the +B treatment (25 mg kg^{-1}) at Rådde, due to higher grass and clover content, respectively.

All the forage mixtures studied here met the demand for Ca, K, Mg, P, Mn and Fe in lactating and dry cows, but not for Na and Cu (Table 3). Zinc was sufficient for dry cows, but not for lactating cows. The dicot weed fraction at Borrarp, dominated by yarrow, had highest concentration of Zn and was the only fraction that fulfilled the lactating cow requirements. However, yarrow can negatively affect the taste of the milk (Spörndly 2003). Since nutrient concentrations in plants vary between plant parts and phenological stages (Lindström et al. 2014), excess of tasty forage allows the animals to sort out the most suitable pieces (Suttle 2010).

On trial level, the slurry fertilisation Borrarp+S decreased the total clover content by 7%, increased K and decreased the Ca, Cu and Mn concentrations in the forage mixture compared with Borrarp-S. In a Danish study Ca, S, Cu and B concentrations in the forage mixture decreased, due to diminished proportion of legumes as a result of slurry application, which favoured the grasses (Pirhofer-Walzl et al. 2011).

Conclusions

In the ley crops investigated, B fertilisation changed the botanical composition and increased the white clover percentage in the forage mixtures. It also increased B concentration in soil, plant tissue of individual plant types and in forage mixtures. Boron application did not affect the concentrations of other elements in the plants and did not affect ley biomass yield. Boron concentrations in the more B demanding legumes, represented by red and white clover, showed normal values in the non B treatment. Under the actual growing conditions, B was not identified as a limiting factor for the ley crops.

From a fodder perspective, there is at present no lower limit in diet recommendations for B, which means a risk for chronical diseases, since there is increasing evidence for B as an important element in animal and human nutrition. Our results showed B concentrations, around 10-fold higher in clover than in grass, which is an advantage for clover with respect to B supply. For other elements in the forage mixtures, the fodder requirement of lactating and dry dairy cows were met for Ca, K, Mg, P, Fe and Mn, and nearly met for S. Zinc was adequate for dry cows, but not for lactating cows and the concentrations of Na and Cu were too low.

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