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Biological nitrogen fixation in three long-term organic and conventional arable crop rotation experiments in Denmark

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ABSTRACT

Biological nitrogen (N) fixation (BNF) by legumes in organic cropping systems has been perceived as a strategy to substitute N import from conventional sources. However, the N contribution by legumes varies considerably depending on legumes species, as well as local soil and climatic conditions. There is a lack of knowledge on whether the N contribution of legumes estimated using short-term experiments reflects the long-term effects in organic systems varying in fertility building measures. There is also limited information on how fertilizer management practices in organic crop rotations affect BNF of legumes. Therefore, this study aimed to estimate BNF in long-term experiments with a range of organic and conventional arable crop rotations at three sites in Denmark varying in climate and soils (coarse sand, loamy sand and sandy loam) and to identify possible causes of differences in the amount of BNF. The experiment included 4-year crop rotations with three treatment factors in a factorial design: (i) rotations, i.e. organic with a year of grass-clover (OGC), organic with a year of grain legumes (OGL), and conventional with a year of grain legumes (CGL), (ii) with (+CC) and without (−CC) cover crops, and (iii) with (+M) and without (−M) animal manure in OGC and OGL, and with (+F) mineral fertilizer in CGL. Cover crops consisted of a mixture of perennial ryegrass and clover (at the sites with coarse sand and sandy loam soils) or winter rye, fodder radish and vetch (at the site with loamy sand soil) in OGC and OGL, and only perennial ryegrass in CGL at all sites. The BNF was measured using the N difference method. The proportion of N derived from the atmosphere (%Ndfa) in aboveground biomass of clover grown for an entire year in a mixture with perennial ryegrass and harvested three times during the growing season in OGC was close to 100% at all three sites. The Ndfa of grain legumes in both OGL and CGL rotations ranged between 61% and 95% depending on location with mostly no significant difference in Ndfa between treatments. Cover crops had more than 92% Ndfa at all sites. The total BNF per rotation cycle was higher in OGC than in OGL and CGL, mostly irrespective of manure/fertilizer or cover crop treatments. There was no significant difference in total BNF between OGL and CGL rotations, but large differences were observed between sites. The lowest cumulated BNF by all the legume species over the 4-year rotation cycle was obtained at the location with sandy loam soil, i.e. 224–244, 96–128, and 144–156 kg N ha^{-1} in OGC, OGL and CGL, respectively, whereas it was higher at the locations with coarse sand and loamy sand soil, i.e. 320–376, 168–264, and 200–220 kg N ha^{-1} in OGC, OGL and CGL, respectively. The study shows that legumes in organic crop rotations can maintain N_2 fixation without being significantly affected by long-term fertilizer regimes or fertility building measures.

1. Introduction

The demand for organically produced grains, either as a food for human consumption or as a feed in livestock production, is increasing in industrialized countries (Yussefi, 2008). Nitrogen (N) is typically the most important yield limiting factor for organic crop production (Olesen et al., 2007), so the N management is one of the greatest challenges for intensification of organic cropping systems (Cassman, 1999; Doltra and Olesen, 2013). In European countries, there is a limitation in use of animal manure from conventional origin in organic farming, and in Denmark it may be completely forbidden by 2021 (Alrøe and Halberg, 2008; Oelofse et al., 2013). In such a context, developing an organic arable farming system, which is self-sufficient in N supply while maintaining high yields, is of utmost importance, but also challenging.

Legumes are an integral part of an organic cropping systems because

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they supply N through biological N fixation (BNF). Legumes can therefore support intensification of organic crop production. Legumes can also improve soil structure and enhance soil biological activity, which support crop nutrient supply (Watson et al., 2002). However, the choice of legume species, soil and climatic conditions and the design of the rotation in an organic system can influence the amount of N contributed by legumes to the cash crops and the effect on crop yields (Doltra et al., 2011).

White and red clover (*Trifolium repens* L. and *Trifolium pratense* L.) are frequent components of green manure crops. The proportion of N derived in clover from BNF (%Ndfa; N derived from the atmosphere) can be up to 98% when the clover is grown in mixture with grass species (Nyfeler et al., 2011). In an organic cropping system experiment with low N fertilization in Switzerland, grass-clover contributed around 120–140 kg N ha^{-1} $year^{-1}$ through BNF (Oberson et al., 2013). Experiments with a range of legume species in mixture with ryegrass in Denmark showed that the annual N contributions from BNF varies from about 160 kg N ha^{-1} for white clover to more than 300 kg N ha^{-1} for red clover or alfalfa (Rasmussen et al., 2012). However, the cost of introducing green manure crops may not be economically justified, since the yield benefits from better soil N supply may not outweigh the extra land needed for the green manure crop (Olesen et al., 2002). This can change as the potentially mineralizable soil organic N and the microbial biomass N pool builds up over time (Petersen et al., 2013). Whether this is sufficient to maintain yield levels in the long-term without external N input still needs to be investigated (Connor, 2013).

Growing a grain legume crop instead of a green manure crop can provide a solution to the loss of cash crop yield from allocating a growing season to the green manure crop. Grain legumes, such as pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.), in the rotation can have a significant residual N effect to the succeeding non-legume crops (Mayer et al., 2003; Hauggaard-Nielsen et al., 2009a). Pea intercropped with cereals in organic systems can fix 41–100 kg N ha^{-1} (Hauggaard-Nielsen et al., 2009b). There has been growing interest in using faba bean in crop rotations (Jensen et al., 2010). Data compiled by Jensen et al. (2010) from several studies showed that faba bean can fix 71–211 kg N ha^{-1} with the mean of 153 kg N ha^{-1} in European conditions. The N fixation by faba bean can save 30–120 kg fertilizer N ha^{-1} for succeeding cereal crops (McEwen et al., 1990; Wright, 1990). This is achieved through low exploitation of soil indigenous N pool and the addition of N to the soil organic matter pools in addition to the harvested grain N (Schwenke et al., 1998). Provided that the soil mineral N is not too high, a successful faba bean crop can fix substantial amount of N_2 (Peoples et al., 2009). In addition, reduced net soil N exploitation by the grain legume can help in building soil fertility. A study by Rochester et al. (1998) suggested that at least 2 Mg DM ha^{-1} in biomass yield is required before significant BNF by faba bean is evident. Negative soil N balance due to the substantial amounts of N withdrawn in grain has also been reported (Patriquin, 1986). Inclusion of rhizodeposition of N during the calculations of BNF, plant available forms of N and climate and soil conditions influence the estimated N_2 fixation and N balance after faba bean crops (Rochester et al., 1998; Crews and Peoples, 2004).

In regards to organic farming, grain legumes are vulnerable to diseases and pests and are also sensitive to climatic stresses such as drought which can affect crop growth (López-Bellido et al., 2005; Saucke et al., 2009) and ultimately also the N fixing ability of the crop. Results from short-term experiments on quantification of BNF by grain legumes species cannot directly be used for quantification of N inputs in organic production systems, since long-term changes in abiotic and biotic factors may affect legume growth and %Ndfa, and such effects may even be enhanced by site-specific conditions and subject to large inter-annual variation.

Nitrogen inputs to the system through BNF using only single year of green manure or grain legumes may not realistically provide sufficient N for a multi-year crop rotation (Mueller and Thorup-Kristensen, 2001). Therefore, inclusion of leguminous cover crops between the main crops in the rotation can be an option to increase N supply to the system. An experiment in Denmark showed that clover as a cover crop can substitute 70 kg N ha^{-1} of animal manure input from the second year of its inclusion in cereal production system compared to ryegrass as a cover crop (Askegaard and Eriksen, 2008). The same study also reported that sole crop of ryegrass as a cover crop was the most effective in reducing nitrate (NO_3^-) leaching, but a clover cover crop also maintained leaching at low level. A vigorous cover crop of grass-clover can have a fertilizer N replacement value of around 100 kg N ha^{-1} in mineral fertilizer for a following spring barley (Askegaard and Eriksen, 2007). In addition, a grass-clover mixture is more efficient in capturing soil N and reducing NO_3^- leaching than a pure clover crop (Loiseau et al., 2001; Askegaard and Eriksen, 2008). The amount of N input in fertilizer and manure will affect the amount of BNF both through the abundance of legumes in grass-legume mixtures and through the proportion of N fixed by the legume plants (Rasmussen et al., 2012). Also, the BNF of legume-based cover crops are highly dependent on how well they are established and this is subject to great inter-annual variation and influenced by method of cover crop establishment and duration of growth in autumn (Doltra and Olesen, 2013).

Soil and climatic conditions which changes over time and additional factors like weeds, diseases and pests, which affect legume biomass production, have considerable influence on total BNF (Rochester et al., 1998; Jensen et al., 2010). When there is abundant soil mineral N available, legumes do not need to fix N_2 to fulfill their N requirement resulting in lower %Ndfa (Schwenke et al., 1998; Jensen et al., 2010). Organic cropping systems in sites with coarse textured soils often have lower available mineral N than in fine textured soils (Petersen et al., 2013), so the soil texture may have an effect on N availability to the legumes and N_2 fixation. Marriott and Wander (2006) has studied the relation between soil types and soil organic carbon in organic and conventional farming but there is limited information on its effect on N_2 fixation in long-term organic systems. Compared to conventional farming, the external N supply (e.g., usually only from animal manure) is usually lower in organic farming (Dawson et al., 2008), so the N_2 fixation can be higher than in conventional systems (Oberson et al., 2013). However, soil available P and other essential nutrients may get low in the long run in organic farming (Gosling and Shepherd, 2005), resulting in adverse effects on BNF (Leidi and Rodriguez-Navarro, 2000; Høgh-Jensen et al., 2002). Effect of difference in climatic conditions on soil organic matter build up has been documented (Marriott and Wander, 2006), but its effect on N_2 fixation has rarely been studied. Studies have argued that organic systems are more tolerant to climatic stress such as drought (Seufert et al., 2012). However, during such stress organic legumes can be outcompeted by weeds and severely affected by diseases. In addition, conventional systems allow early establishment of the cover crop due to the chemical weed control, whereas mechanical weed control may be needed in autumn to control perennial weeds, which reduces options for early establishment of cover crops and thus low contribution of cover crops to the N balance of the system.

A large number of studies have quantified BNF of whole-year green manure, grain legumes and cover crops. However, less is known about how these different legume crops with different growth durations and within different crop combinations/rotations contribute to the total BNF in organic arable farming with low or no external N input. We expect that the lower nutrient availability as well as greater occurrence of diseases and pests in organic systems may enhance variability in BNF compared to conventional systems. Also little is known about how different management practices and soil and climatic conditions will affect BNF by different types of legumes in such systems. We quantified BNF in a range of organic and conventional (fertilized and treated with herbicide and pesticide) arable crop rotations under different soil and climatic conditions in Denmark to better understand which factors affect BNF at the cropping system level. The hypotheses of the current