Soil Biology & Biochemistry

Sources of Nitrogen for Winter Wheat in Organic Cropping Systems

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In organic cropping systems, legumes, cover crops (CC), residue incorporation, and manure application are used to maintain soil fertility, but the contributions of these management practices to soil nitrogen (N) supply remain obscure. We examined potential sources of N for winter wheat (*Triticum aestivum* L.) in four experimental cropping systems established in 1997 on three soil types. Three of the four systems were under organic management. Topsoil N, depth of the A horizon, and cumulated inputs of N since 1997 were determined at plot level. Labile soil N pools [mineral N, potentially mineralizable N (PMN), microbial biomass N (MBN)] were monitored during two growth periods; at one site, biomass C/N ratios were also determined. Soil for labile N analysis was shielded from N inputs during spring application to isolate cumulated system effects. Potentially mineralizable N and MBN were correlated across all sites and rotations (r^2 = 0.72). The MBN corresponded to 46 to 85, 85 to 145, and 74 to 172 kg N ha^{-1} at the three sites and differed significantly between cropping systems, but MBN could not explain differences in wheat grain N yields. Instead, a multiple linear regression model explained 76 and 82% of the variation in grain N yields in organic cropping systems in 2007 and 2008, showing significant effects of, respectively, topsoil N, depth of A horizon, cumulated inputs of N, and N applied to winter wheat in manure. Thus, soil properties and past and current management all contributed to winter wheat N supply.

Abbreviations: CC, cover crops; DM, dry matter; LF-OM, light fraction organic matter; MBC, microbial biomass C; MBN, microbial biomass nitrogen; PMN, potentially mineralizable nitrogen; SIR, substrate-induced respiration.

The extent and timing of soil N mineralization is critical for cereal yields in organic cropping systems. Management strategies to sustain soil N supply include the use of leguminous green manures, cover crops during autumn–winter, incorporation of crop residues following harvest, and judicious use of livestock manure (Olesen et al., 2007). Following 18 yr with annual incorporation of cereal straw at different rates, Thomsen and Christensen (2004) found that soil carbon (C) and N storage increased linearly with straw rate. Constantin et al. (2010) found a positive relationship between C and N inputs via cover crop biomass and soil C and N contents after 13–17 yr in crop rotations on three different soil types. Long-term field experiments also show that plots with annual inputs of animal manure hold more soil C and N than plots receiving mineral fertilizers only (Christensen and Johnston, 1997). Hence, there are cumulative effects of cropping systems with abundant organic inputs on soil total N contents that could influence levels of plant-available N and thus yields of cereal crops. In contrast, residual effects of N added with mineral fertilizers are negligible (Petersen et al., 2010).

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Many studies have investigated labile soil N as an index of crop N supply, for example, to predict the need for N fertilization (Keeney and Bremner, 1966; Raison et al., 1987; Carpenter-Boggs et al., 2000; Mulvaney et al., 2001). Various extraction procedures, as well as mineralization of soil N under laboratory conditions, have been used to assess the size of labile soil N pools (e.g., Schomberg et al., 2009). In a study with archived soils, the N mineralization potential of manure-amended soil was found to increase linearly with the cumulative input of N in manure during a 25-yr period (Whalen et al., 2001), but there is probably no simple link between indices of soil N mineralization potential and N uptake in field-grown crops (Walley et al., 2002; Luxhøi et al., 2007). Ensuring a sufficient flow of plant available N from soil organic N pools to the growing crop remains a challenge in organic cropping systems with cereals such as bread wheat (Cavigelli et al., 2008; Thomsen et al., 2008, 2011). To optimize the design of such cropping systems, identification of the most important source(s) of N for cereal crops would be a major step forward.

Inputs of organic matter have a shorter-term direct and a longer-term indirect effect on the capacity of a soil to deliver plant-available N (Christensen, 2004). During the initial decomposition phase, organic inputs increase microbial activity, which can be associated with net N mineralization or immobilization depending on the C/N ratio and other characteristics of the input (Trinsoutrot et al., 2000). Subsequently, the N residing in residues of the initial decomposition phase adds to the main soil organic N pools, from where N is mineralized at a comparatively slow rate. The cumulative indirect effect of organic inputs thus reflects the duration of the specific management, as indicated above, while the direct effect depends on the quantity, as well as the quality, of recent inputs. To better synchronize soil N mineralization with crop demand, the relative importance of short- and long-term effects of organic inputs needs to be known in greater detail.

This study examined soil N stocks and pools of labile N in soil under winter wheat grown in three different organic cropping systems and one conventionally managed system without organic inputs as reference. Soil sampling took place in two successive years within the four experimental cropping systems at three (2007) and two (2008) sites with different soil types. We hypothesized that residue inputs from legumes and cover crops, and animal manure, would have similar long-term effects on N availability at all sites and that soil N availability and uptake by winter wheat would depend on the specific cropping system. As indicators of N availability, three pools of labile N (mineral N, potentially mineralizable N, and soil microbial biomass N) were determined during the main growth phase (April–August) of winter wheat.

MATERIALS AND METHODS

Experimental Sites and Cropping Systems

This study took place in the third cycle (2005–2008) of long-term field experiments initiated in 1997 on three soil types with cereal-based four-crop rotations (Olesen et al., 2000). The three experimental sites were a coarse sandy soil (Orthic Haplohumod) at Jyndevad in Southwest Denmark, a loamy sand (Typic Hapludult) at Foulum in Western Denmark, and a sandy loam (Typic Agrudalf) at Flakkebjerg in Eastern Denmark. The soils were characterized in 1996 by 16 randomly taken samples to a 1-m depth in all field plots (Olesen et al., 2000). Depth of the A horizon and total N in the plow layer was determined as described previously (Djurhuus and Olesen, 2000). Site details are given in Table 1. The clay content ranged from 55 g kg^{-1} at Jyndevad to 155 g kg^{-1} at Flakkebjerg where, however, the clay content was highly variable, ranging from 110 to 200 g kg^{-1} at

Table 1. Geographical location, temperature and precipitation (means 1961–1990), and selected soil characteristics for the three experimental sites (Djurhuus and Olesen, 2000; Olesen et al., 2000).

Location		Jyndevad	Foulum	Flakkebjerg
		54°54′ N lat, 9°08′ E long	56°30′ N lat, 9°34′ E long	55°19′ N lat, 11°23′ E long
Texture class		sand	sandy loam	sandy loam
Coarse sand (200–2000 mm)	g 100 g^{-1}	73.1	27.2	22.9
Fine sand (20–200 mm)	g 100 g^{-1}	18.0	47.0	47.4
Silt (2–20 mm)	g 100 g^{-1}	2.4	13.3	12.4
Clay (<2 mm)	g 100 g^{-1}	4.5	8.8	15.5
Soil organic matter	g 100 g^{-1}	2.0	3.8	1.7
Soil organic C	g 100 g^{-1}	1.17	2.29	1.01
Depth of A horizon				
Minimum	mm	268	348	366
Maximum	mm	341	929	723
Total N	g 100 g^{-1}	0.085	0.175	0.107
Bulk density	g cm^{-3}	1.47	1.41	1.56
Mean annual temperature				
2007	°C	9.6	8.8	9.6
2008	°C	9.5	8.8	9.5
Mean annual precipitation				
2007	mm	1107	668	660
2008	mm	917	723	554

the field plot level (Schjønning et al., 2012). The content of soil organic matter was least at Flakkebjerg despite the higher clay content, presumably as a result of previous decades of intensive arable crop cultivation with little organic inputs.

Table 2. The four cropping systems employed in this study. All crops of all four rotations were present each year at each of the three experimental sites. O2+CC, O4+CC, and O4−CC are organic cropping systems with or without cover crops, while C4−CC is a conventional reference.

Rotation	O2+CC	O4+CC	O4−CC	C4−CC
Crop 1	spring barley:ley	spring barley^{CC} †	spring barley	spring barley
Crop 2	grass-clover	faba bean^{CC}	faba bean	faba bean
Crop 3	potato	potato	potato	potato
Crop 4	winter wheat^{CC}	winter wheat^{CC}	winter wheat	winter wheat

† CC, cover crop; at Jyndevad and Foulum a mixture of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), and red clover (*Trifolium pratense* L.), at Flakkebjerg a mixture of winter rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth), and fodder radish (*Raphanus sativus* L.).

Three organic cropping systems were selected for this study, together with one conventionally managed system that was, until 2004, managed without fertilizer inputs; the crop sequences are shown in Table 2. Every crop was represented each year in two completely randomized blocks. The cropping systems addressed three management factors: the proportion of leguminous crops, the inclusion of cover crops, and the N source used for fertilization. The organic system O2+CC included a grass-clover crop as well as cover crops. Grass-clover cuts were left to decompose in the field during the two first cycles of the experiment (1997–2004) but removed from 2005 onward. All other crop residues were left in the field. An organic cash crop rotation was represented with and without cover crops (O4+CC and O4−CC, respectively). The fourth rotation (C4−CC) was identical to O4−CC, but was managed with mineral fertilizers and pesticides.

The cover crops at Jyndevad and Foulum were a mixture of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.), and red clover (*Trifolium pratense* L.). These were undersown in cereals and pulses in spring. The cover crops at Flakkebjerg were a mixture of winter rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth), and fodder radish (*Raphanus sativus* L.) sown in autumn after harvest and stubble cultivations to control perennial weeds. Further details on field management and soil properties are reported elsewhere (e.g., Chirinda et al., 2010; Askegaard et al., 2011).

The organic systems received a rotational mean of 70 kg total-N ha^{-1} yr^{-1} in pig slurry applied during late March to mid-April. The slurry was surface-applied to winter wheat as a top-dressing using trail hoses. Slurry composition varied somewhat between years and sites. In 2007, slurry dry matter (DM) content was 2.1 to 3.0%, and winter wheat received 103 to 113 kg total-N ha^{-1} of which NH_4^+–N constituted 70 to 76%. In 2008, the slurry dry matter content was 2.5 to 5.2%, and winter wheat received 101 to 102 kg total-N ha^{-1} of which NH_4^+–N constituted 74 to 82%.The C4−CC rotation was top-dressed with 158 to 168 kg N ha^{-1} in calcium ammonium nitrate, with one third in late March and the rest about 1 mo later.

Table 3 presents average annual above-ground C and N inputs for each site and cropping system for the periods 1997–2004 (first two rotations) and 2005–2007 (this rotation). Field plots used for C4−CC were managed without N fertilization (and pesticides) from 1997 until 2004, when this cropping system was converted to conventional management; hence, this system was initially less fertile than the three other cropping systems.

Table 3. Average annual input of carbon (Mg C ha^{-1}) and nitrogen (kg N ha^{-1}) from aboveground residues, manure, and fertilizer applied during 1997–2004 (previous rotations) and from 2005 until the year before sampling, and average grain N yield in winter wheat (*Triticum aestivum* L.) in 2007 and 2008 (kg N ha^{-1}).

Location	O2+CC	O4−CC	O4+CC	C4−CC
Carbon input (1997–2004)				
Jyndevad	3.21b †	3.02b	3.15b	2.26a
Foulum	4.16c	2.96b	4.50d	2.11a
Flakkebjerg	3.72c	2.86b	3.89c	1.85a
Nitrogen input (1997–2004)				
Jyndevad	147b	141b	145b	92a
Foulum	185c	104b	187c	45a
Flakkebjerg	155c	105b	161c	39a
Carbon input (2005–2007)				
Jyndevad	0.37a	1.36b	1.80c	1.39b
Foulum	1.82a	2.08ab	2.30b	2.11ab
Flakkebjerg	1.96b	0.93a	1.14a	1.05a
Nitrogen input (2005–2007)				
Jyndevad	62a	106b	132c	131c
Foulum	123a	124a	147b	167c
Flakkebjerg	133b	79a	92a	114ab
Grain N yield (2007–2008)				
Jyndevad	32.2a	24.8a	31.4a	102.9b
Foulum	86.4a	71.2a	84.7 a	167.5b
Flakkebjerg	54.9a	46.6a	49.4a	157.0b
Grain DM yield (2007–2008)				
Jyndevad	2.32a	1.84a	2.15a	5.67b
Foulum	5.04a	4.37a	4.92a	8.30b
Flakkebjerg	3.61a	3.17a	3.41a	6.92b
Grain N concentration (g N kg^{-1})				
Jyndevad	14.4a	13.6a	14.4a	18.4b
Foulum	16.4a	15.4a	16.2a	19.9b
Flakkebjerg	15.3a	14.7a	14.6a	22.7b

† Values followed by different letters within a row are significantly different ($P < 0.05$).

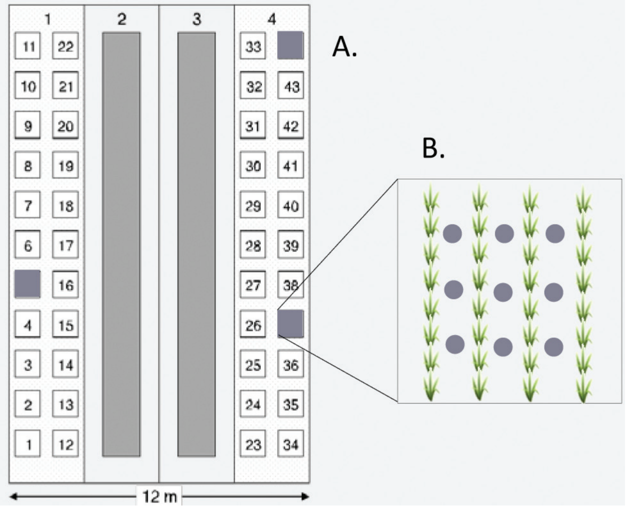

Fig. 1. Outline of field plot showing the basic design (dimensions vary between sites). (a) Two central harvest strips are flanked by strips with miniplots for individual studies, three of which were randomly selected among miniplots with no prior treatments. (b) Using a template, cylinders to be recovered during the growing season were installed between crop rows shortly after seeding of winter wheat (*Triticum aestivum* L.) in autumn.

Each field plot (8 × 15 m^2) was split into four sections: two sections for harvest flanked by two sections with 21 to 44 (depending on site) 1 × 1 m^2 miniplots for separate studies (Fig. 1a). Three miniplots were randomly selected in field plots under winter wheat. With two experimental blocks, a total of 24 miniplots at each experimental site were thus sampled every year for monitoring of labile soil N pools as specified below.

Field Sampling in 2007

In preparation for the 2007 soil sampling, stainless steel cylinders (volume 250 cm^3, height 8.6 cm) were inserted between crop rows shortly after plant emergence in November 2006. This approach was taken to minimize any interference from crop roots. A total of nine cylinders were distributed as shown in Fig. 1b; these were installed using a special adaptor leaving the cylinders approximately 10 mm below the soil surface. During fertilization in the spring 2007 (trail hose application of pig slurry) the inter-row soil was covered by 1-m lengths of split PVC pipe to allow uniform fertilization of the crop without exposing the soil in the cylinders for sampling to fresh inputs of N.

Two cylinders were retrieved from each miniplot on 10 Apr. 2007, 21 May 2007, 2 July 2007, and 14 Aug. 2007; sampling positions had been randomized at the time of installation. Emerging weeds were removed by hand at sampling, but if a weed had established inside a cylinder to be sampled, the ninth cylinder was used instead. The cylinders with the soil cores were transported to the laboratory in a cooler and stored overnight at 2°C before being processed as described below. Winter wheat was harvested at physiological maturity in late August (i.e., after the last soil sampling) and grain yields determined.

Field Sampling in 2008

In 2008, field sampling took place in other field plots where winter wheat was grown this year. The Jyndevad site was not sampled in 2008. Instead, we studied the effect of roots on labile N pools (which was negligible; data not shown) by sampling in paired miniplots. The sampling dates in 2008 were 2 April, 14 May, 24 June, and 8 August. At each sampling, cylinders from identical positions in the two paired miniplots were sampled and the soil cores pooled for analysis. Methods for sample handling were as in 2007. All visible roots were removed when soils were sieved and mixed. Harvest was in August, shortly after the last soil sampling.

Processing of Soil Samples

The two soil cores of each miniplot were combined, sieved and mixed, and subsampled for analyses (Fig. 2). Ten-gram subsamples were extracted end-over-end for 30 min in 40 mL 1 *M* KCl for determination of NH_4^+, NO_3^-, and ninhydrin-reactive N in filtered extracts. Other 10-g subsamples were used to determine gravimetric soil water content. A third set of 10-g soil subsamples was weighed into 30-cm^3 glass beakers, transferred to desiccators and fumigated with ethanol-free chloroform (Amato and Ladd, 1988). After 7 d, chloroform was removed from the fumigated soil samples, and the soils extracted with KCl, as described above for ninhydrin-reactive N, to determine MBN. Potentially mineralizable N was determined according to Keeney and Bremner (1966): 30-g subsamples were transferred to 100-mL flasks and amended with 20 mL deionized water. The flasks were then closed with septum and screw cap, evacuated, and refilled with N_2. Any residual oxygen remaining in the soil would have been rapidly consumed when the flasks were incubated at 40°C. After 7 d at 40°C, the soils were supplied with 30 mL 2 *M* KCl, mixed vigorously by hand, and then extracted in

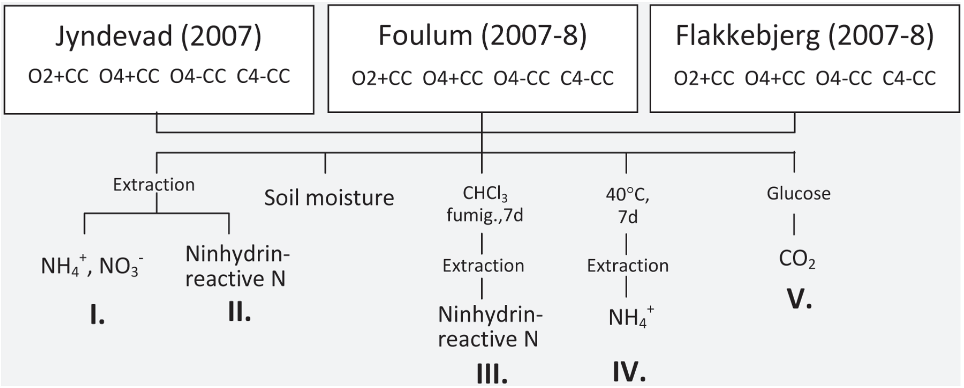

Fig. 2. Analytical flow scheme for the samples retrieved from the four cropping systems at the three experimental sites. A total of 24 individual samples from each site was processed for determination of soil mineral N (I), microbial biomass N based on ninhydrin-reactive N released after chloroform fumigation (II, III), microbial biomass C based on substrate induced respiration (V), and potentially mineralizable N based on an anaerobic incubation at 40°C (IV).

horizontal position on a shaker for 30 min. After filtration, the extracts were analyzed for NH_4^+. Finally, at Flakkebjerg a set of 2-g soil samples was used for determination of microbial biomass C (MBC) using substrate-induced respiration (SIR). Soil samples were placed in 117-mL serum bottles and amended with 5 mL of a solution containing 20 mg mL^{-1} glucose. Bottles were capped, mounted on a shaker, and CO_2 concentrations in the headspace measured 0.5 and 4 h after sealing.

Analytical Methods

Soil NH_4^+ and $NO_2^- + NO_3^-$ in filtered 1 *M* KCl extracts was determined colorimetrically using an AutoAnalyzer 3 system (Bran+Lübbe, Norderstedt, Germany) and standard colorimetric methods (Keeney and Nelson, 1982). Ninhydrin-reactive N was determined on 1-mL subsamples of the KCl extract as previously described (Petersen et al., 2002). Soil-specific conversion factors were not determined. Instead the value of 3.1 proposed by Amato and Ladd (1988) was adopted to calculate MBN from ninhydrin-reactive N.

For analysis of CO_2 in the SIR assay, 0.5 mL gas samples were injected on a gas chromatograph with thermal conductivity detector and a 1.8 m × 3 mm Haysep column (Mikrolab, Højbjerg, Denmark). Respiration rate (mg CO_2–C kg^{-1} soil h^{-1}) was multiplied by 76 h to get to MBC (mg C kg^{-1} soil; Anderson and Domsch, 1978).

Grain dry matter and grain N were determined by a near-infrared spectroscopy analyzer (Infratec 1241 Grain Analyzer, Foss A/S) as described by Buchmann et al. (2001).

Data Analysis

Microbial biomass nitrogen, PMN, and biomass C/N ratios were analyzed statistically using a repeated measures mixed model with year, site, cropping system, and sampling time as fixed effects and including all two-way interactions. The covariance structure used to account for auto-correlation between sampling times was ARH(1). Random effects of block and system × block were included in the model. Different subsets of the data were analyzed separately, i.e., effects of cropping system on MBN and PMN were tested across all three sites (2007 only), effects on MBN and PMN across both years were tested using results from Foulum and Flakkebjerg, and effects of cylinder insertion time on MBN and PMN were tested using Flakkebjerg results from 2008. Finally, effects on biomass C/N were evaluated for Flakkebjerg, the only site where both MBC and MBN was determined.

Average annual inputs of C and N in above-ground residues and animal manure returned to the soil during the periods 1997–2004 and 2005–2007, and grain N yields in 2007 and 2008, were analyzed by site using a mixed model with year and block as random effects.

To further explore sources of plant available N, MBN and grain N yields in the three organic cropping systems (O2+CC, O4+CC, and O4−CC) were related to initial topsoil N (in 1996), average annual input of N in above-ground plant residues and manure, NH_4^+–N in manure applied to winter wheat in 2007 and 2008, depth of the A horizon, and year using multiple linear regression. Since all treatments received very similar rates of pig slurry, the regression analysis would not allow a robust estimate of the effect of manure N. Instead the coefficient for effect of NH_4^+–N in manure was a priori set at 0.42 kg grain N kg^{-1} NH_4–N in accordance with the average N recovery of winter wheat recorded in the field experiment (Olesen et al., 2009). All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC) and resulting effects evaluated based on the differences of least square means.

RESULTS

Field Sampling 2007

At all three sites, and in all four cropping systems, soil mineral N concentrations were generally <10 mg kg^{-1} (Fig. 3). Microbial biomass N represented the largest pool of labile N, accounting for 17–31, 31–54, and 23–53 mg N kg^{-1} at Jyndevad, Foulum, and Flakkebjerg, respectively. Microbial biomass N was significantly ($P < 0.01$) smaller at Jyndevad than at the other two sites, where concentrations were similar. The concentrations of potentially mineralizable N differed significantly between all three sites ($P < 0.001$). Within each site, the O2+CC system always had the highest ($P < 0.05$) and C4−CC the lowest ($P < 0.01$) concentration of both PMN and MBN. At the level of cropping system, both PMN and MBN concentrations were always higher at Foulum than at Jyndevad. The concentrations at Flakkebjerg and Foulum were similar except for system C4−CC. Cropping systems with cover crops had higher levels of both PMN and MBN at Flakkebjerg compared to Jyndevad. The temporal dynamics of the different labile N pools during the 2007 growing season were small, although with a general increase in MBN ($P < 0.001$) between the second sampling in May and the third sampling in late June.

Field Sampling 2008

In 2008, field sampling at the Jyndevad site was discontinued. The sampling of cylinders installed in November was repeated at Foulum and Flakkebjerg. Microbial biomass N again represented the largest pool of labile N, with 31–42 mg N kg^{-1} at Foulum and 23–48 mg N kg^{-1} at Flakkebjerg. While the overall patterns among cropping systems were also evident in 2008, the differences were generally smaller than in 2007 (Fig. 4). When data from Foulum and Flakkebjerg were analyzed across both years, the concentrations of PMN in Foulum soil were significantly higher than at Flakkebjerg for each of the four systems ($P < 0.05$). In contrast, soil MBN concentrations were not significantly different between the two sites for any of the four systems. At Flakkebjerg, concentrations of PMN and MBN in the two cropping systems without cover crops (C4−CC and O4−CC) were similar when based on data from 2 yr, and this was also true when comparing O4−CC and O4+CC. All other contrasts were significantly different ($P < 0.05$). Although treatment effects were more complex at Foulum, concentrations of PMN

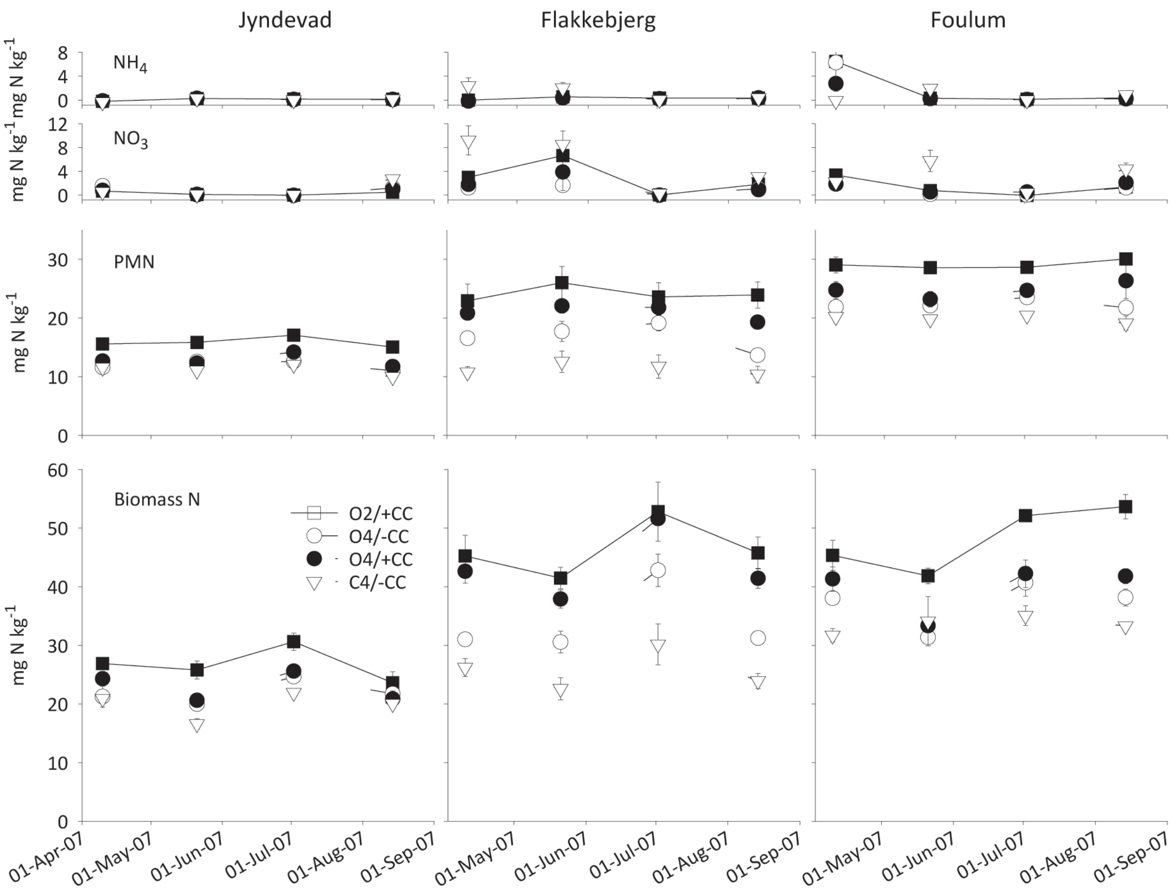

Fig. 3. Labile pools of N in soil from the four cropping systems on three soil types (Jyndevad, Flakkebjerg, Foulum) during growth of winter wheat (*Triticum aestivum* L.) in 2007. Cylinders (250 cm^3) were installed immediately below the soil surface in November the previous year; these samples were shielded during application of manure and fertilizer to isolate long-term system effects. CC, cover crops; MBN, microbial biomass N; PMN, potentially mineralizable N.

and MBN in O2+CC remained significantly above those of the other systems.

Microbial Biomass Carbon and MBC/MBN Ratios

Soil samples from Flakkebjerg were subsampled for determination of MBC in both years (Fig. 5). In 2007, levels of MBC were higher and more dynamic in organic cropping systems compared to the less fertile C4−CC system. In 2008, the differences in both concentration range and seasonal dynamics were smaller. Based on MBN and MBC results, MBC/MBN ratios were calculated (Fig. 6). Significant effects of year ($P < 0.01$) and sampling time ($P < 0.001$) were identified by the mixed model. Across both years and all cropping systems, MBC/MBN increased ($P < 0.05$) between the first two samplings in April and May, respectively. Across both years, MBC/MBN ratios of the last sampling in early August differed significantly ($P < 0.001$) from those of the other three samplings.

Sources of Grain Nitrogen Yield

The yields of grain N in C4−CC were significantly higher than those obtained in the three organic cropping systems (Table 3), reflecting the higher inputs of plant available N in mineral fertilizers. The higher grain N yield in C4−CC compared to the organic systems was a consequence of higher dry matter yield in combination with higher grain N concentration. Grain N yields of the three organic cropping systems were not statistically different, although yields in O2+CC were always slightly higher, and those in O4−CC slightly lower, than yields obtained in O4+CC. These trends were consistent with the relative differences observed for PMN and MBN (Fig. 3), but there was no significant relationship between MBN and grain N yield within individual sites (data not shown). A multiple linear regression analysis showed that MBN depended significantly on total N concentration in the topsoil ($P < 0.01$), the depth of the A horizon ($P < 0.05$), and annual N inputs since 1997 ($P < 0.05$), but not year. Nitrogen applied in manure to winter wheat in 2007 and 2008 was not included in this analysis since the soil samples collected for analysis were covered during slurry application.

A further regression analysis linked yields of wheat grain N to the factors mentioned above and in this case also to the slurry N given to wheat in the organic treatments in the respective growing seasons. The cumulated input of N in above-ground residues and manure since 1997 varied between 0.94 and 1.95 Mg N ha^{-1}, with the smallest values at Flakkebjerg and the largest at Foulum (Table 3). The amount of N at a 0- to 25-cm depth varied between 2.75 and 7.11 Mg N ha^{-1}, with the smallest values at Jyndevad and the largest at Foulum. The amount of NH_4^+–N applied to winter wheat with pig slurry in 2007 and 2008 varied between 76 and 88 kg N ha^{-1}. The statistical model in Table 4 also included a response to depth of the A horizon, which varied between 27 and 93 cm, being most shallow at Jyndevad and with considerable variation at both Foulum and Flakkebjerg (Table 1). Again the effect of year was not significant, and the regression model was rerun without this variable. The remaining variables were all strongly significant (Table 4), and a good overall agreement was obtained between measured and predicted grain N yields (Fig. 7a). There was no colinearity between the independent variables (all condition indices <2). The estimated response to average annual N inputs since 1997 was nearly twice as high as to soil N. This model, developed for the three organic cropping systems, was subsequently used for predicting N yield of the conventionally managed system, C4–CC (Fig. 7b). The N use efficiency of mineral N was set to 0.48 as an average for early application of N fertilizer in field experiments with winter wheat in Denmark (Olesen et al., 2003). The model based on sources of N in the organic rotations underestimated grain N yields in C4–CC by on average 74 kg N ha^{-1}.

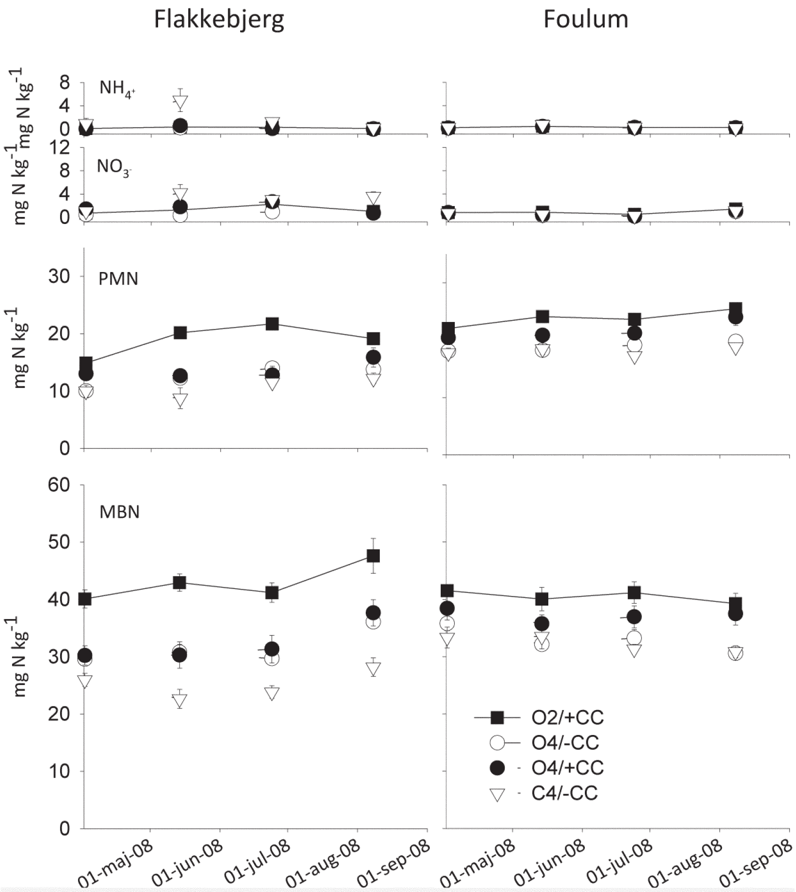

Fig. 4. Labile pools of N in soil from the four cropping systems on two soil types (Flakkebjerg, Foulum) during growth of winter wheat (*Triticum aestivum* L.) in 2008. Cylinders (250 cm^3) were installed immediately below the soil surface in November the previous year. CC, cover crops; MBN, microbial biomass N; PMN, potentially mineralizable N.

DISCUSSION

This study examined sources of plant available N in arable soil as influenced by site, cropping system, and management. Soil total N stocks and cumulated N inputs at the plot level were quantified, and labile N pools were monitored during growth of winter wheat. To emphasize long-term effects of cropping system, soil sampling took place in winter wheat where disturbances due to tillage and residue incorporation had not occurred since the previous autumn. Effects of spring fertilization and crop N uptake were eliminated by installing cylinders at the time of seeding in autumn and by shielding the soil to be sampled from direct inputs of mineral fertilizer and slurry. Consequently, the concentrations of mineral N in the soil sampled were small throughout the study (Fig. 3 and 4) and will not be discussed further.

Pools of Labile Organic Nitrogen

We characterized the availability of soil N by potential, rather than actual N mineralization rates, since in situ soil coring techniques have been reported to be less reliable in long-term studies (Hanselman et al., 2004). Instead, PMN was quantified by an assay based on 7 d of anaerobic incubation at 40°C (Keeney and Bremner, 1966). Myrold (1987) compared this PMN assay with chloroform fumigation for a range of forest soils and concluded, based on ^{15}N-labeling experiments, that the source of PMN was almost entirely microbial biomass. In our study of arable loamy soils there was, across all three soil types, a strong correlation between PMN and MBN concentrations irrespective of the cropping system (Fig. 8). With an intercept significantly above but close to zero, a common origin is indicated. However, MBN was calculated from ninhydrin-reactive N using a conversion factor of 3.1 and, consequently, PMN > ninhydrin-reactive N, suggesting that some nonmicrobial N was mineralized during the PMN assay. Yu et al. (2011) reported a strong correlation ($r = 0.93$) be-

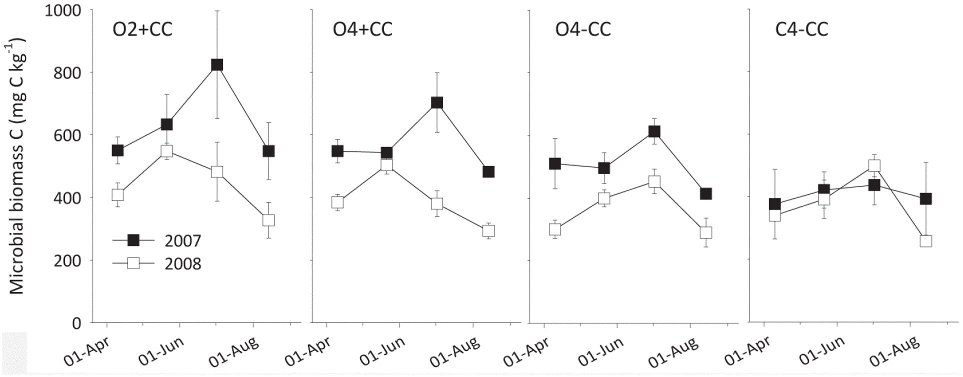

Fig. 5. Microbial biomass C determined in 2007 and 2008 at Flakkebjerg using substrate induced respiration. CC, cover crops.

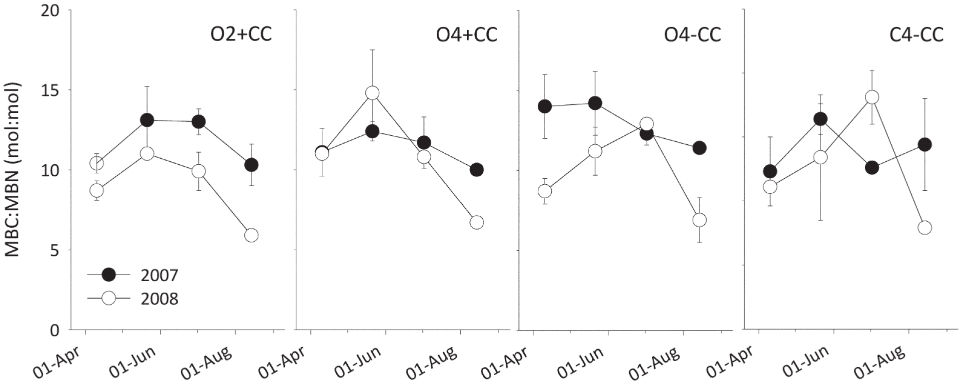

Fig. 6. Microbial biomass C/N ratios at Flakkebjerg calculated for each of the four cropping systems in 2007 and 2008. CC, cover crops; MBC, microbial biomass C; MBN, microbial biomass N.

tween N released by chloroform fumigation and plant uptake of N, but numerically the plant uptake was greater than five times higher than the amounts of N extracted after fumigation. Hence, there may be a significant potential for mineralization of N in soil organic matter in arable soils. In the present study, plant-available N was supplied in pig slurry or mineral fertilizer, which may explain the moderate seasonal dynamics of PMN and MBN. On the other hand, similar dynamics were observed by Franzluebbers et al. (1994) for unfertilized soil under wheat.

Site and System Effects on Labile Organic Nitrogen

At all three sites there was a consistent pattern across cropping systems with respect to levels of PMN and MBN; O2+CC with grass-clover and cover crops always showed the highest values of both PMN and MBN, particularly at Foulum and Flakkebjerg (Fig. 3 and 4). Biederbeck et al. (1994) reported that the frequency of plant cover in a rotation was positively correlated with several indices of labile organic matter, including mineralizable N, biomass N, and N in light-fraction organic matter (LF-OM, <1.9 g cm^{-3}). Also, Wander and Traina (1996) found that a rotation with cover crops had a larger proportion of the soil C and N in LF-OM compared with two other rotations without cover crops. Given the different proportions of cover crops in our four cropping systems, LF-OM and the associated microbial biomass may have been partly responsible for the differences in PMN and MBN.

Crop rotations and general field management were identical at the three experimental sites, yet there were large site-related differences in labile N pools. The Flakkebjerg site with the highest clay content showed the widest range in both PMN and MBN, while the coarse sandy soil at Jyndevad had the narrowest range. Clay minerals may, directly or indirectly, protect microorganisms against predation (Six et al., 2006), and differences in soil clay content could play a role for these site related differences. However, Kanazawa and Filip (1986) found that concentrations of bacteria, actinomycetes, and fungi were up to 100-fold higher for particulate organic matter than for a silt-clay fraction. Part of LF-OM in arable soils is occluded in aggregates, and this proportion could increase with soil clay content (Gregorich et al., 2006). Protection of LF-OM via aggregate formation may thus also contribute to differences in labile N pools between sites and cropping systems.

Dynamics of Labile Organic Nitrogen

Based on average bulk densities (Table 1) and assuming that the soil sampled represents the plow layer, MBN ranged from 46 to 85 kg N ha^{-1} at Jyndevad, from 85 to 145 kg N ha^{-1} at Foulum, and from 74 to 172 kg N ha^{-1} at Flakkebjerg. And as indicated by the PMN vs. MBN relationship discussed above, there were also nonmicrobial sources of mineralizable N. Hence, labile organic N pools in arable soil are far from negligible. There were consistent trends in MBC/MBN ratios during the growing season (Fig. 6), but these trends were dominated by changes in MBC concentration (Fig. 5), whereas changes in MBN were moderate (Fig. 3 and 4). Nevertheless, labile pools of N may be actively involved in soil N transformations (Cookson et al., 2005). For example, Luxhøi et al. (2007) found that long-term annual inputs of cereal straw and organic manure increased the gross N mineraliza-

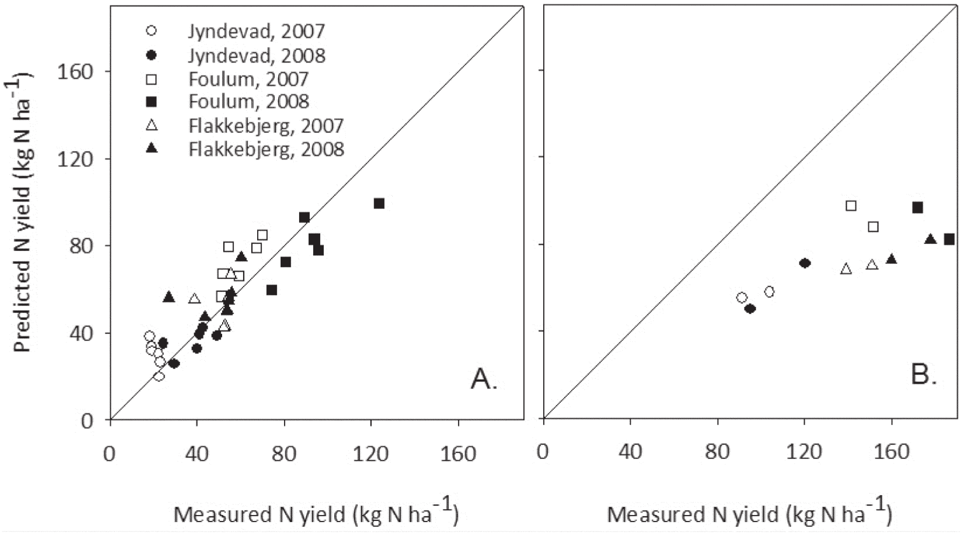

Fig. 7. (a) Grain N yields in winter wheat (*Triticum aestivum* L.), predicted using the statistical model summarized in Table 4, plotted against measured grain N yields for each combination of site and year. The analysis included only the three organic cropping systems (O2+CC, O4+CC, and O4–CC). (b) Grain N yields in winter wheat in C4–CC, predicted using the model derived from organic rotations summarized in Table 4; an N use efficiency of 0.48 was assumed for N in mineral fertilizer.

tion-immobilization turnover rate without influencing net N mineralization rate.

Gregorich et al. (2006) identified LF-OM as the primary source and sink of N in arable soil, but with a C/N ratio of nearly 20 (including the associated microbial biomass) the importance of LF-OM for crop N supply was questioned. Likewise, Christensen (2001) concluded that LF-OM, rather than being a source of mineral N, is a source of readily available C and energy for decomposers and that turnover of LF-OM may well be linked to net N immobilization. With fresh organic inputs, the N mineralization-immobilization turnover will depend on substrate quality and C/N ratio (Trinsoutrot et al., 2000).

The C/N ratio of the microbial biomass varied between 5 and 15 (Fig. 6), a range slightly wider than reported for microbial biomass in soils with organic amendments in a recent meta-analysis (Kallenbach and Grandy, 2011). Interestingly, their analysis found no relationship between the C/N ratio of the organic amendment and the C/N ratio of the microbial biomass, and they concluded that the physiological requirements of microorganisms determined cell composition. This implies that seasonal dynamics in MBC/MBN (Fig. 6) may reflect successional changes in the microbial populations, for example, in the proportions of fungal and bacterial biomass. Organic cropping and the use of cover crops are management options that may increase fungal/bacterial ratios (Six et al., 2006). In accordance with this, Christensen et al. (2012), studying the same set of soil samples from Flakkebjerg, found higher proportions of fungal biomass in the three organic cropping systems (O2+CC, O4−CC, O4+CC) than in the conventional system (C4−CC), based on both SIR inhibition and nematode trophic groups. Seasonal changes were not identified by Christensen et al. (2012), but the late-spring increase in MBC/MBN ratios could have reflected a relative increase in the proportion of fungi with a C/N ratio of 10 to 15 against a ratio of 4 to 6 for bacteria (Six et al., 2006; Wallenstein et al., 2006). To the extent that labile N was associated with microbial biomass in a LF-OM fraction with a wide C/N ratio, the potential for net N immobilization via microbial growth during spring could explain the limited dynamics in labile N pools observed.

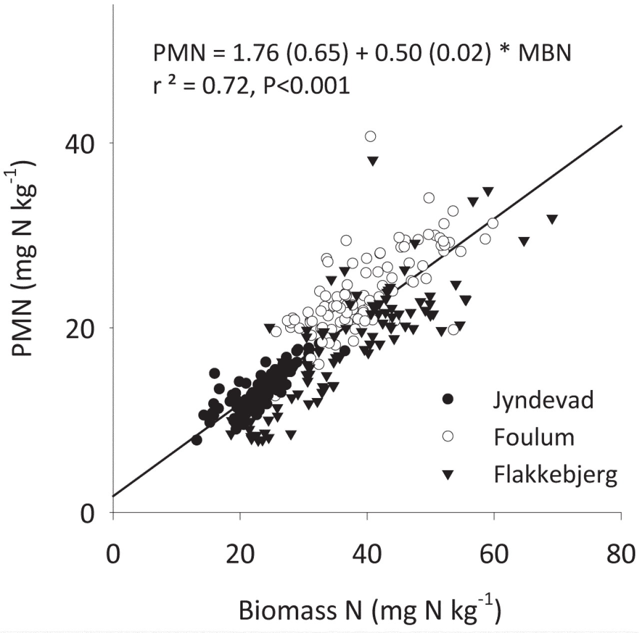

Fig. 8. Potentially mineralizable N (PMN) plotted against microbial biomass N (MBN). The regression equation represents samples from all three sites and both years.

Sources of Nitrogen for Winter Wheat

Grain N yields in cropping system C4−CC responded strongly to mineral N fertilizer application (Table 3). The higher grain N yields in C4−CC was a result of higher dry matter yields, as well as higher grain N concentrations. The organic cropping systems received only around 65% of the N input given to the conventional system to reflect the fact that arable organic farms typically have limited access to livestock manure (ICROFS, 2008). This shortage may be partly compensated by the inclusion of cover crops. According to a recent study analyzing N uptake by spring cereals in the O2 and O4 rotations at Jyndevad, Foulum, and Flakkebjerg, the positive effect of cover crops on DM yields tended to increase over time (Doltra and Olesen, 2013).

Even though the pools of labile N differed considerably between the three organic cropping systems, wheat grain N yields were not significantly different (Table 3). Alternative sources of N for the winter wheat crop were, therefore, explored by a multiple linear regression model to evaluate effects of soil N content in the plow layer and below, as well as historical and current N inputs (Table 4). In this model, NH_4^+–N was preferred to total N since manure is stored for up to 1 yr and, therefore, is well-degraded at the time of application, as indicated by proportions of NH_4^+ of around 80%. This aligns with the prescribed first year availability of 75% for pig slurry (Petersen and Sørensen, 2008). The models indicated that soil properties, crop rotation, and management all contributed significantly to the availability of N to winter wheat grown in the organic cropping systems. A substantial part of the variation was clearly related to site (Fig. 7a). Also, grain N yields were consistently higher in 2008 than in 2007. But even within year and site, there was generally a positive relationship between predicted and measured yields (Fig. 7a),

Table 4. Regression of winter wheat grain N yield (kg N ha^{-1}) based on different factors for plots managed under organic farming (rotations O2+CC, O4+CC, and O4−CC) (R^2 = 0.75, RMSE = 12.7, n = 36). The parameter estimates, the standard error of the estimates (SE) and the probability values are shown. The partial R^2 values (R_p^2) are based on the SAS type II SS corresponding to squared Pearson correlation coefficients when effects of other variables are not accounted for.

Parameter	Estimate	SE	P	R_p^2
Intercept	−97	13	0.0001	
Total N in topsoil (top 25 cm) (kg N ha^{-1})	0.010	0.0017	0.0001	0.52
N applied since 1997 (kg N ha^{-1} yr^{-1})	0.030	0.0079	0.0006	0.31
Manure N applied to wheat (kg NH_4–N ha^{-1})	0.42	–	–	–
Depth of A horizon (cm)	0.49	0.141	0.0015	0.27

suggesting that grain N yields were determined by a combination of soil and management factors. This result is in accordance with a recent meta-analysis by Ros (2012), who found that organic N extractable after aerobic or anaerobic incubation did reflect the N supplying power of the soil, but the predictive power was low due to large and unexplained differences in regression coefficients, that is, soil properties alone could not account for crop N supply.

When the model derived from the three organic cropping systems was applied to predict grain N yield in the conventionally managed cropping system (C4–CC), there was still a strong positive relationship between measured and predicted yields, although grain N yields were underestimated by on average 74 kg N ha^{-1}. This amount is similar in magnitude to the negative intercept of −97 kg N ha^{-1} in the equation shown in Table 4. Factors such as competition from weeds (Olesen et al., 2009), effects of diseases (Olesen et al., 2003), and limitation of nutrients other than N (Askegaard et al., 2003) may all contribute to the negative intercept. Furthermore, the same recovery in grain N yield was assumed for N inputs in each of the past 10 experimental years, but most likely recent inputs of N contributed relatively more. Hence, some of the deviation between predicted and measured grain N yield in C4–CC (Fig. 7b) may be due to the fact that N inputs to this system were concentrated in the period 2005 to 2007 (cf., Table 4) and, therefore, made a higher than average contribution to crop N supply.

The results suggest that it may be possible to predict crop N supply for cereals in organic arable cropping systems by combining information on soil properties with recent management history. These observations also provide valuable insights into how soil fertility building supports crop yields. Such information may be used in the development of decision support tools for both short- and long-term N management in organic farming systems.

CONCLUSIONS

This study quantified potential sources of N for winter wheat in four cropping systems at three sites. We found consistent, though site-specific differences in labile organic N pools between cropping systems differing in their proportions of overwintering crops, manure, and fertilizer application. The microbial biomass was the main reserve of labile N, but dynamics during the growing season was moderate and not directly related to crop yields. To explain grain N yields a combination of factors, including soil N content, depth of the A horizon, inputs of N in previous years, and the N fertilization in a given year, were required. Thus, inherent soil properties and past and current management all contributed to winter wheat N yields.

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