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Cover crops prevent the deleterious effect of nitrogen fertilisation on bacterial diversity by maintaining the carbon content of ploughed soil

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ABSTRACT

Synthetic nitrogen (N) fertilisers are widely used for enhancing agrosystem productivity and are thus thought to increase organic inputs from crop residues. However, many crop rotations have a low amount of organic residue returned to the soil since the whole aboveground crop biomass is harvested and exported. To compensate for such organic outputs and to improve soil quality, the introduction of winter cover crops in rotations has been suggested. A 4-year controlled field experiment was conducted to quantify the respective and combined effects of chemical N fertilisation and winter cover crops on plant productivity, organic carbon (C) and N inputs from crop residues and cover crops, changes in soil C and N concentrations, C:N ratio, soil mineral N, pH, soil moisture and soil bacterial biodiversity. A ploughing tillage system with low organic input was assessed, for which the main crops were spring wheat, green pea, forage maize, along with cover crops of different legume and non-legume species.

N fertilisation did not have an impact on the aboveground biomass except following forage maize. Cover crops increased the total amount of C and N inputs, irrespective of N fertilisation which had no significant effect. The soil N concentration decreased in all treatments, particularly when N fertilisers were applied under bare fallow conditions. The latter treatment also caused decreased soil C concentrations (slightly increased in the other treatments) and decreased bacterial biodiversity (no change in the other treatments). Bacteria from the Proteobacteria and Bacterioidetes phyla were highly correlated with soil from fertilised bare fallow conditions. While Verrucomicrobia was characteristic of non-fertilised bare fallow soils, Acidobacteria and Cyanobacteria were associated with the high C and N concentrations present in soils following cover crop treatments.

Taken together, these results demonstrate that in ploughing systems, under low organic restitution regimes, intensive N fertilisation decreases the diversity of the bacterial soil community and reduces soil C and N concentrations, but only in bare fallow conditions. There is a protective effect of winter cover crops against the deleterious effect of chemical N fertilisation on soil biodiversity and nutrient cycling, since they can maintain soil C and N concentrations. The use of winter cover crops containing legumes is thus a practice that is able to meet the criteria of a sustainable agriculture.

1. Introduction

Several studies have reported that there is a decline in soil organic carbon (C) content worldwide and especially in Europe (Capriel, 2013; Heikkinen et al., 2013). Nevertheless, most cultivated soils are still tilled using the moldboard ploughing technique (Higashi et al., 2014; Triplett and Dick, 2008), which is known to provoke deleterious effects on both soil carbon content and on soil living organisms (Kladivko, 2001; Jacobs et al., 2009; Leite et al., 2009; Nyamadzawo et al., 2009). An increase in the soil organic C pool can be obtained by the use of cover crops, which provide additional C through better energy conversion during crop rotation (Sainju et al., 2007; Smith et al., 2014). In addition, cover crops improve aboveground biodiversity (Balota et al., 2014; Calderon et al., 2016) and provide substantial amounts of N when they are composed of legume species (Kramberger et al., 2014). In conventional crop cultivation systems, the increase in crop productivity by mineral N fertilisers can lead to an increase in N and C inputs into the soil (Sainju et al., 2002, 2006;

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Bakht et al., 2009; Mazzoncini et al., 2011), depending on the type of crop rotation. Intensive N fertilisation can also promote soil organic matter (SOM) mineralisation through modifications of soil microbe communities (Jenkinson et al., 1985; Kuzyakov et al., 2000; Kuzyakov, 2010; Majumder and Kuzyakov, 2010). However, the impact of fertilisers on soil microbial diversity is complex because it involves several factors such as the type and the amount of fertiliser applied, as well as the nature of the soil and the crop rotation (Lupwayi et al., 2012). Generally, N fertilisation induces a loss of microbial diversity and modification of the composition of the bacterial community (Ramirez et al., 2010; Coolon et al., 2013; Cederlund et al., 2014; Willekens et al., 2014; Zhao et al., 2014).

The impact of N fertilisation (Shen et al., 2010; Gomez and Garland, 2012; Zhao et al., 2014; Sun et al., 2015; Zhou et al., 2015; Zeng et al., 2016) and the use of winter cover crops (Carrera et al., 2007; Liu et al., 2007; Nair and Ngouajio, 2012) have not been studied previously in an integrated manner. In particular, soil C sequestration, soil N content and microbial diversity in ploughing-based agricultural systems have received little attention. We thus developed a field experiment to quantify the individual and synergistic effects of cover crops and chemical N fertilisation on the soil microbial community and soil C and N contents under tillage conditions. We found that over a short period of time, intensive N fertilisation reduced both soil C and N contents. We also observed that under high N fertilisation input, the diversity of the soil microbial community decreased. In addition, we found that the detrimental effect of intensive N fertilisation on both soil properties and soil microbial communities can be substantially reduced when using a cover cropping system.

2. Materials and methods

2.1. Site description and experimental design

The field experiment was conducted at the experimental site of La Woestyne, in North France (50°44′N, 2°22′E, 40 m a.s.l.). The average annual air temperature and total rainfall were 10.5 °C and 675 mm, respectively, with amounts of rainfall relatively homogeneous across seasons. Soil particle size composition was characterized by silt 66.8%, clay 21.2% and sand 12%. The concentrations of organic C, total N and SOM before the beginning of the experiment were 13.1 g kg^{-1}, 1.5 g kg^{-1} and 22.5 g kg^{-1} respectively (see Table 1 for more characteristics).

Prior to the start of the experiment in 2009, the field was managed using a chisel plough and rotary power system. A crop rotation method for which organic restitution was known for being low and weakly affected by N fertilisers was employed to highlight the effect of N fertiliser on biodiversity and soil C and N concentrations (Fig. 1). It consisted of spring wheat (*Triticum aestivum* L.) in 2010, followed by green peas (*Pisum sativum* L.) in 2011, maize (*Zea mays* L.) in 2012, and spring wheat in 2013. In order to study the effect of fertilisation and cover crops on biodiversity and chemical parameters, the experimental field was split into four treatments with three replicate plots for each: bare fallow without (BFN0) or with (BFNX) N fertilisation; winter cover crops without (CCN0) or with (CCNX) N fertilisation. BFN0 and CCN0 plots measured 7 × 8 m, while BFNX and CCNX plots measured 14 × 8 m. The cover crops consisted of a mixture of three legumes and three non-legume species, which were sown as follows: 400 seeds m^{-2} of Egyptian clover (*Trifolium alexandrinum* L.), 30 seeds m^{-2} of faba bean (*Vicia faba* L.), 50 seeds m^{-2} of vetch (*Vicia sativa* L.), 80 seeds m^{-2} of flax (*Linum usitatissimum* L.), 200 seeds m^{-2} of phacelia (*Phacelia tanacetifolia* Benth.), 60 seeds m^{-2} of oat (*Avena sativa* L.). Before the main crops were sown, cover crops were buried by a conventional moldboard plough to a depth of 30 cm. Spring wheat was sown at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) combined with a rotating harrow and crop residues were returned to the soil. Green pea was sown at a row spacing of 17 cm using a Turbosem drill (Herriau, France) combined with a rotating harrow. According to European regulations, pea did not receive N fertilisation in NX treatments. Crop residues of green pea were returned to the soil. Maize was sown at a row spacing of 75 cm with a Maxima drill (Kuhn, France) following a rotating harrow. Maize was grown for silage, meaning that all the aboveground residues were removed from the field. The dose of N fertiliser applied in the NX treatments was determined according to the N budget method for maize (108 kg N ha^{-1}) and wheat (160 kg N ha^{-1}). N fertilisation was applied in the form of urea for both maize and wheat and crop protection was ensured conventionally.

Table 1
Main characteristics of the 0–10 cm soil layer before the beginning of the experiment in 2009.

Parameters (units)	Mean ± SE
pH in H_2O	6.28 ± 0.160
CEC[a] (cmol + kg^{-1})	11.67 ± 0.272
P_2O_5[b] (g kg^{-1})	0.13 ± 0.008
Organic C (g kg^{-1})	13.08 ± 0.042
Total N (g kg^{-1})	1.48 ± 0.004
SOM (g kg^{-1})	22.50 ± 0.007
Soil C:N ratio	8.85 ± 0.105

SOM: soil organic matter.
SE: standard error of the mean.
[a] Cation-exchange capacity (Metson method).
[b] Available phosphorus (Olsen method).

2.2. Total biomass and C and N inputs from main crops and cover crop residues

During the experiment, a 3 × 1 linear meter row of the main crop was sampled each year at the time of harvest, in each plot. The part of the plant that is commonly harvested (i.e., grains) was separated from the rest of the aboveground biomass (i.e., crop residues incorporated into the soil). Samples were oven-dried at 65 °C for 3 days and subsequently weighed (± 0.1 g accuracy) to determine total aboveground biomass. Each sample was then ground into a powder prior to C and N analysis using an elemental analyser (Flash EA 1112 series, Thermo Electron, Germany).

For winter cover crops, 3 × 1 m^2 was sampled each year in each plot, just before ploughing. Samples were oven-dried at 65 °C for three days and subsequently weighed. The total aboveground biomass was determined and then ground into a powder prior to C and N analysis. C and N inputs for each treatment since the beginning of the experiment were measured as the sum of the amounts of C and N in the crop residues plus the C and N in cover crops since 2009.

2.3. Soil sampling and chemical analyses

In late March 2013, 1 month after the last ploughing, 11 months after the last N application and >1 year after the last cover crop incorporation, six 10 cm deep soil cores were randomly collected using a 2 cm diameter auger in each of the three replicate plots. Samples 10 cm deep were chosen since previous studies indicated that it was an appropriate depth for the assessment of the effects of N additions on microbial communities (Coolon et al., 2013; Zeng et al., 2016), even in ploughed soils (Sun et al., 2015). The six soil cores were composited together into a single sample. Soils were then sieved using a 2 mm mesh and divided into two parts: the first one was stored at 4 °C to await chemical analysis, and the second one was stored at −20 °C until DNA extraction.

The soil moisture concentration was determined by oven-drying at 105 °C. Soil pH was measured using a pH meter FE20-FiveEasy™ (Mettler Toledo, Switzerland) at a ratio of 1:5 (weight/volume) of soil to distilled water, following shaking for 45 min. Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were extracted at a ratio of 20 g fresh soil to 100 mL 1 M KCl. After shaking for 1 h, the extracts were centrifuged for 10 min at 4000 rpm, and the supernatants were analysed by a continuous flow analytical system (San^{++} system, Skalar, Holland). Total