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Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla

Harald Cederlund [a], Ella Wessén [a], Karin Enwall [a], Christopher M. Jones [a], Jaanis Juhanson [a], Mikael Pell [a], Laurent Philippot [b,c], Sara Hallin [a,*]

[a] Swedish University of Agricultural Science, Department of Microbiology, Box 7025, 75007 Uppsala, Sweden
[b] INRA, UMR1347 Agroecologie, 2100 Dijon, France
[c] Université de Bourgogne, UMR 1229, F-21000 Dijon, France

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ABSTRACT

Agricultural practices affect the soil ecosystem in multiple ways and the soil microbial communities represent an integrated and dynamic measure of soil status. Our aim was to test whether the soil bacterial community and the relative abundance of major bacterial phyla responded predictably to long-term organic amendments representing different carbon qualities (peat and straw) in combination with nitrogen fertilization levels and if certain bacterial groups were indicative of specific treatments. We hypothesized that the long-term treatments had created distinctly different ecological niches for soil bacteria, suitable for either fast-growing copiotrophic bacteria, or slow-growing oligotrophic bacteria. Based on terminal-restriction fragment length polymorphism of the 16S rRNA genes from the total soil bacterial community and taxa-specific quantitative real-time PCR of seven different groups, all treatments significantly affected the community structure, but nitrogen fertilization was the most important driver for changes in the relative abundances of the studied taxa. According to an indicator species analysis, the changes were largely explained by the decline in the relative abundances of Acidobacteria, Gemmatimonadetes and Verrucomicrobia with nitrogen fertilization. Conditions more favourable for copiotrophic life strategies were indicated in these plots by the decreased metabolic quotient, i.e. the ratio between basal respiration rate and soil biomass. Apart from the Alphaproteobacteria that were significantly associated with peat, no taxa were indicative of organic amendment in general. However, several significant indicators of both peat and straw were identified among the terminal restriction fragments suggesting that changes induced by the organic amendments were mainly manifested at a lower taxonomical level. Our findings strengthen the proposition that certain higher bacterial taxa adapt in an ecologically coherent way in response to changes induced by fertilization.

1. Introduction

Agricultural soils are managed with the aim to maintain or increase productivity, which is typically achieved by sustaining a high soil organic matter (SOM) content and applying nitrogen (N) fertilizers. Both mineral nitrogen and organic amendments affect the size and composition of soil bacterial communities, as well as the ecosystem processes and services they mediate, e.g. soil organic matter decomposition, nutrient cycling and carbon sequestration. Agricultural practices that impact SOM can significantly alter the indigenous soil microbial community structure (Enwall et al., 2007; Peacock et al., 2001; Stark et al., 2008), and it has been suggested that the bacterial community structure is more affected than the fungal community by organic fertilizers (Lejon et al., 2007; Marschner et al., 2003). In addition, several recent studies have shown that N-fertilization leads to changes in the relative abundances of specific bacterial phyla (Nemergut et al., 2008; Turlapati et al., 2013; Wessén et al., 2010). Ramirez et al. (2012) demonstrated that some of these changes appear to be consistent across a broad range of soil types and that Actinobacteria and Firmicutes were consistently favoured by N-fertilization, whereas the relative abundance of Acidobacteria and Verrucomicrobia decreased.

Philippot et al. (2009, 2010) argued that high bacterial taxa display properties of ecological coherence since they respond predictably to environmental factors, although ecological traits that characterize bacterial taxa defined at higher taxonomic ranks may be more unifying than universal. Earlier, Fierer et al. (2007)

* Corresponding author. Tel.: +46 18 67 32 09; fax: +46 18 67 33 92.
E-mail address: sara.hallin@slu.se (S. Hallin).

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proposed that certain bacterial phyla could be differentiated into the more ecologically relevant copiotrophic and oligotrophic categories based on their substrate preferences and life strategies, and that applying this classification framework may aid in the interpretation of bacterial community dynamics using taxonomic data. Predictable responses of certain bacterial taxa also open up the possibility to use them as indicators of instance the trophic status of soils (Hartman et al., 2008; Smit et al., 2001). The prospect of using indicator organisms for soil status is tractable since they capture dynamic properties, and biological indicators have gained renewed interest (Pulleman et al., 2012; Ritz et al., 2009) not least because of the emerging European Soil Framework Directive.

The aim of this study was to test whether long-term addition of organic materials differing in carbon quality combined with or without addition of mineral N-fertilizers have created distinctly different ecological habitats for soil bacteria and if bacterial taxa responded predictably to trophic status or C quality. For this purpose, soil was sampled from a field trial that had been fertilized for 46 years with continuous amendments of straw or peat, with or without addition of mineral-N, and an unfertilized control. Our hypothesis was that the N-fertilized soils in particular, with their higher primary production, should be environments more suitable for fast-growing copiotrophic bacteria, whereas the unfertilized plots should be more favourable for slow-growing oligotrophic bacteria. The abundances of typically dominant soil bacterial phyla were quantified and the overall bacterial community structure was determined along with the soil microbial substrate use efficiency and respiration.

2. Materials and methods

2.1. Field site, soil sampling and chemical analysis

Soil was sampled from the Ultuna long-term soil organic matter experiment in Uppsala, Sweden (Kirchmann et al., 1994 Fig. S1). It was established in 1956 with a block design, where each of the four blocks comprises 15 treatments in plots of 2×2 m separated by wooden frames. One of the blocks does not have randomly distributed plots and was omitted from the study. For this study, five treatments replicated in three blocks were selected (Table 1): straw (*S*), straw and N (SN), peat (*P*), peat and N (PN), and the unfertilized control (UC). Nitrogen was added as $Ca(NO_3)_2$ yearly in spring at a rate corresponding to 80 kg N ha^{-1}. The soils with organic amendments received 8000 kg ha^{-1} ash free organic matter as straw or peat every second fall in even-numbered years. As an average since 1956, the added straw and peat contained 900 and 985 mg organic matter and 6.6 and 7.8 m g N g^{-1} dry matter, respectively (Kirchmann et al., 1994). All treatments have been fertilized yearly with 22 kg phosphorus (P) and 35–38 kg potassium chloride ha^{-1}. The plots have been under the same crop rotation, and since 2000 have been planted with maize (*Zea mays*) of the same cultivar each year. Soil was sampled in August 2002 directly after harvest, but before the organic amendments to maximize the time period between organic amendments and fertilization in order to minimize potential short-term effects of the treatments. From each plot, 10 soil cores (2 cm diameter, 20 cm depth) were collected between plant rows, mixed into one composite sample per plot and sieved (4 mm mesh width) before storage at −20 °C until analysis.

Soil properties and crop yield were obtained from the monitoring program of the site (The Department of Soil and Environment, Swedish University of Agricultural Sciences; Table 1). The soil is a clay loam, classified as a Eutric Cambisol. Total nitrogen (Tot-N) was measured as Kjeldahl-N (ISO 13,878), soil organic carbon (SOC) was determined by dry combustion (ISO 10,694), pH was measured in water, and water holding capacity (WHC) was determined gravimetrically. Crop yield was determined as dry weight of total harvested green biomass, and this value was used to estimate plant primary production. To compensate for year-to-year variations due to weather conditions, we used the average crop yield from 2000 to 2006 to estimate the effect of the treatments on plant primary production (Table 1).

2.2. Basal soil respiration and substrate-induced respiration

Basal soil respiration was determined in duplicate for each plot according to Stenberg et al. (1998) by transferring 20 g of soil to 250 ml respirometric jars, adjusting the WHC to 60% and placing them in a respirometer (Respicond III, Nordgren Innovation AB, Umeå, Sweden). The soil was incubated for eight days at 22 °C. During the incubation, the produced CO_2 was trapped in 10 ml 0.2 M potassium hydroxide solution and the CO_2 concentration was estimated by conductivity readings every 30 min. The subsequent decrease in conductivity was used to calculate the basal respiration rate by linear regression using the data collected during the last 24 h of the incubation. After this incubation, a substrate-induced respiration (SIR) rate was determined after addition of 75 mg glucose, 11.3 mg $(NH_4)_2SO_4$, 3.5 mg KH_2PO_4 and 100 mg talcum powder. The SIR was calculated as the instantaneous rate of CO_2-formation upon addition of the substrate, by non-linear regression of accumulated conductivity data, and thus reflects the size of the microbial biomass prior to glucose addition. A metabolic quotient (qCO_2) was calculated as a ratio between basal respiration and SIR.

2.3. DNA extraction

DNA was extracted in duplicate from a total of 1 g soil using the FastDNA SPIN kit for Soil according to the manufacturer's instructions (MP Biomedicals, Santa Ana USA). The two DNA extracts were pooled before further analysis. The quality and size of the DNA was checked by agarose–gel electrophoresis and quantified using a spectrophotometer at 260 nm.

Table 1
Soil properties and crop yields for the sampled treatments at the Ultuna long-term soil organic matter experiment (mean ± standard deviation, $n = 3$). Different letters after the values indicate treatments with significant differences ($P < 0.05$).

	Treatment	pH[1,3]	SOC[1,2] (% dw)	Tot-N[1,2] (% dw)	C:N[1]	WHC	Crop yield[2,4] (kg dw ha^{-1})
UC	Unfertilized control	6.2 ± 0.1^{ab}	1.3 ± 0.12^{d}	0.13 ± 0.012^{c}	10.3 ± 0.3^{b}	32 ± 0.2^{e}	4080 ± 460^{c}
S	Straw	6.4 ± 0.1^{ab}	1.5 ± 0.02^{d}	0.14 ± 0.002^{c}	10.3 ± 0.1^{b}	33 ± 0.3^{d}	4470 ± 400^{bc}
SN	Straw + N	6.5 ± 0.1^{a}	1.9 ± 0.09^{c}	0.18 ± 0.009^{b}	10.6 ± 0.1^{b}	37 ± 0.5^{c}	8840 ± 410^{a}
P	Peat	5.6 ± 0.3^{c}	3.2 ± 0.06^{b}	0.18 ± 0.002^{b}	17.7 ± 0.3^{a}	39 ± 0.7^{b}	5610 ± 780^{b}
PN	Peat + N	6.0 ± 0.1^{bc}	3.8 ± 0.08^{a}	0.22 ± 0.003^{a}	17.6 ± 0.2^{a}	41 ± 0.6^{a}	10430 ± 490^{a}

[1] When the experimental site was established in 1956, the soil pH was 6.6 and the total organic C and total N were 1.5% and 0.17% of the soil dry weight, respectively.
[2] Abbreviations: dw, dry weight; SOC, total soil organic carbon; Tot-N, total soil nitrogen; C:N, organic C to total N ratio; WHC, water holding capacity.
[3] ($n = 9$).
[4] The crop yield (total above-ground biomass, $n = 21$) is based on the mean maize yield during year 2000–2006.