



Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field



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ARTICLE INFO

Keywords:

Mineral fertiliser

Manure

Nutrients

Soil microbiome

MicroResp

Metabolic fingerprints

ABSTRACT

Applications of mineral and organic fertilisers are an important agricultural practice for improving crop yields, but these applications can also affect soil properties, as well as the microbial community structure and function. The objective of this study was to compare the legacy effects of long-term applications of mineral fertiliser *versus* animal slurry on the composition and activity of soil microbial communities, and to investigate the significance of their application level and change in long-term management.

Soils were collected from the Long-Term Nutrient Depletion Trial in Taastrup, Denmark, which consists of seven mineral and animal slurry fertilisation treatments (20 years) plus seven additional treatments that underwent a shift in their long-term management after 14 years. Microbial communities in soils receiving animal slurry had multiple substrate-induced respiration (MSIR) metabolic fingerprints that clearly differentiated them from those in mineral fertiliser-amended soils. The differences in fingerprints were mainly related to soil total C and pH. Bacterial community analysis by 16S rRNA gene amplicon sequencing showed that animal slurry applications increased bacterial community richness and diversity compared with mineral fertiliser applications, and also increased the relative abundance of several copiotrophic taxa. Even high levels of annual nutrient applications (mineral and organic) promoted copiotroph-dominated bacterial communities to the detriment of oligotrophs, with N inputs exerting a greater influence on bacterial community structure than P or K levels. Stable long-term annual fertiliser applications supported a diverse bacterial community, while a change in nutrient management decreased bacterial diversity. This study documents the differing, time-dependent effects of organic inputs and mineral fertilisation on the composition and functionality of the soil microbial community and highlights the importance of nutrient input levels for soil bacterial community structure.

1. Introduction

Maintenance or even improvement of soil fertility is essential for sustainable agricultural production in the future (Tilman et al., 2002). Routine applications of mineral and organic fertilisers are an important agricultural practice for improving nutrient availability to crops and ultimately crop yields, but to meet the increasing global demand for food it is important to understand how these agricultural management practices affect the soil processes underpinning high soil quality and crop productivity.

Organic inputs, such as animal manure, can increase the soil

contents of nutrients and organic carbon (SOC), and can increase soil pH (Blanchet et al., 2016; Li et al., 2015; Zhong et al., 2009). Inorganic fertilisers can similarly increase nutrient availability, but typically decrease the soil pH (Zhao et al., 2014) as a result of proton release to the soil caused by N cycling (nitrification of applied ammonium) and the acidic reaction of soluble P fertilisers. However, depending on the specific form and composition of the fertiliser, maintenance of (Plaza et al., 2004) or increases in soil pH (Zhong et al., 2009) may also be observed. Furthermore, to counteract an undesirable drop in soil pH, which can be detrimental to crop growth, it is normal agricultural practice for farmers to apply lime.

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Fertiliser regime can be expected to affect the soil microbial biomass, community structure and activity (Bünemann et al., 2006; Böhme et al., 2005; Marschner et al., 2003) either directly or indirectly via increased crop production and subsequent returns as organic matter. In a long-term field experiment, Francioli et al. (2016) found greater bacterial diversity and richness in soils fertilised with farmyard manure than in soils receiving mineral fertilisers. Other long-term field studies support these findings. Li et al. (2015), for example, found that annual application of organic fertiliser increases bacterial community biomass and diversity, while Hartmann et al. (2015) reported increased richness and decreased evenness of bacterial communities in soils under organic management, which was primarily attributed to the use and quality of the organic inputs. Interestingly, bacteria preferring nutrient-rich environments (Proteobacteria and Firmicutes) and bacteria known to be involved in the degradation of complex organic compounds increased in relative abundance in these soils (Francioli et al., 2016; Hartmann et al., 2015). Furthermore, Li et al. (2017) reported that applications of organic manure and mineral fertilisers together increased the abundance of specific taxa involved in the decomposition of complex organic matter and soil nutrient transformation compared with the application of inorganic fertilisers alone.

The observed effects of mineral fertilisation alone on soil microorganisms are often relatively small, but the review of Geisseler and Scow (2014) reported an average 15% increase in microbial biomass C in mineral fertiliser vs. unfertilised control treatments across 64 long-term field trials around the world. Several studies have also identified bacterial groups that are favoured by mineral fertiliser inputs, for example Actinobacteria and Chloroflexi (Chu et al., 2007; Hartmann et al., 2015; Liu et al., 2010). In the study of Hartmann et al. (2015), mineral fertiliser applications resulted in reduced richness and increased evenness of soil microbiota characterised by oligotrophic organisms. Importantly, the reduction in soil pH when ammonium-N-containing fertilisers are applied is a confounding factor that has a considerable influence on the biomass, structure and activity of the soil microbial communities (Francioli et al., 2016; Geisseler and Scow, 2014). Hence soil pH has been identified as an important driver of community changes along with soil organic C and soil N, while soil P may play a more limited role (Francioli et al., 2016; Hartmann et al., 2015).

Soil microorganisms are important for soil fertility due to the role they play in organic matter decomposition, nutrient cycling, formation of organic complexing agents and maintenance of soil structure (Murphy et al., 2007). These functions of soil microorganisms are thought to be linked to their metabolic capabilities (Nannipieri et al., 2003), which can be assessed through metabolic fingerprints based on the amount of respired carbon dioxide (CO₂) following the addition of a range of substrates (Campbell et al., 2003). Furthermore, studies to assess soil microbial diversity and community structure have been greatly facilitated by modern next-generation sequencing methods targeting the 16S rRNA gene. However, to date, only a limited number of studies have exploited this high-throughput technology to determine the effect of fertiliser regimes on soil microbiomes.

It should be noted that the response of the microbial community to fertilisation in the long term may differ from its response in the short term (Allison and Martiny, 2008). The accumulated effect of repeated annual applications may gradually alter the community structure or functionality (Geisseler and Scow, 2014). Long-term experiments in combination with chemical analyses, metabolic fingerprinting and next-generation sequencing are therefore essential to clarify the effects of nutrient applications on the microbial community. However, existing studies combining these approaches often focus on a comparison of management systems (e.g. mineral fertilisers vs. organic amendments) rather than on ranges of specific nutrient input combinations to define threshold concentrations for the fertiliser impacts. Additionally, only a few studies have also considered soil properties and crop yield levels when examining changes in microbial activity and community

structure.

The overall objective of this study was therefore to assess the long-term legacy effects of different mineral and organic fertilisation inputs on the composition and activity of the soil microbial communities. To do so, the study used the Long-Term Nutrient Depletion Trial (LTNDT, Taastrup, Denmark), which includes a broad range of long-term (> 20 years) mineral and organic fertiliser input combinations, as well as combinations where the fertiliser regime has been changed more recently (in 2010). It was hypothesised that: 1) organic fertiliser applications increase bacterial community richness, diversity and function to a greater extent than mineral fertiliser applications, 2) differences in the composition of the bacterial community become more pronounced the longer the treatments are applied, and 3) within the mineral fertilisers, differences in N input level have a greater effect on the structure or function of the bacterial community than P or K inputs.

2. Materials and methods

2.1. Field site and experimental design

The LTNDT was established in 1964 at the Experimental Farm of the University of Copenhagen in Taastrup, Denmark (55°40' N, 12°17' E). Details of the experimental history, design and management practices have been described by van der Bom et al. (2017a), therefore only a brief summary is provided here. The field was cropped with cereals for more than 30 years, receiving 60 kg N ha⁻¹ a⁻¹ in mineral fertiliser, before seven permanent mineral fertiliser (combinations of N, P and K) and organic slurry treatments were introduced in 1995–1996 (Fig. 1). The current design includes a single crop rotation with 14 different input treatments, which resulted from a diversification in 2010. All plots of the seven original treatments were divided into two subplots, and a new treatment was created in one half (by adjusting the N, P or K application rates), while the seven original treatments continued unchanged in the other half. The seven new and diversified treatments thus represent a shift in long-term management, while the seven original treatments continue unchanged.

For the organic fertiliser treatments, slurry was imported each year from farms nearby, with the type varying between either cattle or pig slurry depending on the year (pig slurry from 1996 to 1999, and from 2010 to 2016; and cattle slurry from 2000 to 2008). The slurries contained the majority of their total N in the form of ammonium-N (on average 530 g kg⁻¹ for cattle slurries and 650 g kg⁻¹ for pig slurries), with the remainder entirely in organic form (mainly undigested proteinaceous N) and no content of nitrate or nitrite. The mineral N, P and K fertilisers were applied as ammonium-nitrate fertilisers (i.e. with equal parts of NH₄-N and NO₃-N forms), single or triple-superphosphate, and potassium chloride (KCl). Furthermore, lime (mainly CaCO₃) was added to the entire field in the autumn of 2004 (3 Mg ha⁻¹) and in 2015 (2.5 Mg ha⁻¹).

Average yield levels for the periods 1996–2008 have previously been determined by van der Bom et al. (2017a) and have been determined in the same manner for 2010–2016 (summarised in Table 1). Considering that the diversified treatments were split off from the original seven (continued) treatments in 2010, yield levels in the previous period were identical between each corresponding pair. All treatments were replicated in four blocks with a gross plot size of 50 m × 20 m, giving a total of 56 plots for the whole trial. The soil type is a sandy loam (Luvisol; FAO, 2015) (164 g kg⁻¹ clay, 173 g kg⁻¹ silt, 333 g kg⁻¹ fine sand, 312 g kg⁻¹ coarse sand and 17 g kg⁻¹ organic matter). The long-term average (1960–2008) annual temperature of the location is 7.8 °C and average annual precipitation is 607 mm.

2.2. Soil sampling and analyses

In September 2016, after the harvest of the crop, bulk soil samples (25–30 cores) were taken representatively from the plough layer (0–

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