Soil Biology & Biochemistry xxx (2014) 1–8

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Microbial and microfaunal communities in phosphorus limited grazed grassland change composition but maintain homoeostatic nutrient stoichiometry

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ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form
9 March 2014
Accepted 30 March 2014
Available online xxx

Keywords:
Bacterial:fungal ratio
Bottom-up control
Nematodes
Phosphorus
PLFA
Soil microbial biomass
Top-down control

ABSTRACT

Previous results from a long-term grassland trial, located in south-east Ireland indicated conserved (homoeostatic) nutrient stoichiometry of the soil microbial biomass despite widely varying soil C:N:P ratios. To determine whether this was associated with a change in microbial community structure, rather than a change in microbial physiology, this study characterized the responses of below-ground microbial and nematode community structure to P fertilization. The trial site maintained a range of P fertilisation rates (0–30 kg P ha^{-1} yr^{-1}) which had been applied since 1968 and soil samples were collected in spring 2009, autumn 2010 and spring 2011. The microbial biomass demonstrated homoeostatic stoichiometry over all sampling occasions, particularly of the C:P ratio, despite a 50-fold difference in soil solution C:P ratio. However, Microbial and nematode community structure also varied with P fertilisation, indicating that nutrient ratios are maintained even though there were changes in microbial community structure. P fertilization induced a shift from fungal to bacterial dominated decomposition pathways, as indicated by the proportion of bacterial-feeding to fungal-feeding nematodes and bacterial: fungal phospholipid fatty acids (PLFAs). The altered microbial community structure was considered to result from bottom-up control of nutrient quality and quantity by altered vegetation structure and fertilizer inputs, as well as top-down pressures from the nematode community.

1. Introduction

Phosphorus (P) is one of the least available of all essential nutrients in soil with available concentrations generally below that of many micronutrients (Zvomuya et al., 2006). For example, in a south China latosol available P was approximately 2 mg^{-1} kg^{-1} soil while the available Fe and Mn were 17 and 170 mg^{-1} kg^{-1} soil, respectively (Li et al., 1999). The low solubility of P makes it a common key growth-limiting nutrient in soil and water (Sharpley et al., 2001) such that, globally, low availability of P is a major constraint for crop production in many low-input agricultural systems (Raghothama, 1999). Available phosphorus in Irish grazed grassland soils had declined to concentrations as low as 1 mg kg^{-1} in the early 1940s (Brogan, 2001) limiting plant and therefore animal production. There followed a rapid increase in the use of inorganic P fertiliser in the 1960/70s which stabilized at a national consumption of *c.* 60,000 t yr^{-1} from the early 1980s (Tunney, 1990). Since 1996 there has been a downward trend in P fertilizer use, as P inputs were in excess of outputs and contributed to the eutrophication of surface waters (Foy et al., 1997; Tunney, 2002). However, the increase in available soil P from previous fertilisation was such that in 2000, 50% of Irish grassland soils no longer showed a yield response to added P (Culleton et al., 2002).

The form, dynamics and mobility of P in soil-plant-animal-water systems results from a combination of biological, chemical, physical, and environmental properties and processes, together with the history and intensity of grassland use and management (Condron et al., 1996). The biological component involves microorganisms that require nutrients for their own growth and generally the ratio of carbon (C): P determines whether P is immobilised in the microbial biomass or mineralised to become available for uptake. The soil microbial biomass therefore acts as both a sink and a source of P which becomes available as a result of microbial turnover.

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http://dx.doi.org/10.1016/j.soilbio.2014.03.024
0038-0717/

A number of effects on soil biology have been noted as a consequence of changes in P fertilisation in a long term (instigated in 1968) agricultural trial on grazed Irish grassland (Culleton et al., 2002). Returning to this long-term experimental platform, soils which had received no P since 1968 were characterised by strong microbial P limitation and soils with high P addition by oversaturation of microorganisms with P (Griffiths et al., 2012). Despite large differences in available soil C: N: P ratios, the microbial biomass exhibited stable (homoeostatic) elemental stoichiometry. This stable stoichiometry could result from a shift in microbial community structure or an altered microbial physiology within the same microbial community structure (Tezuka, 1990; Scott et al., 2012). Microbial responses to P fertilisation are variable: in a long-term arable trial, for example, microbial biomass, microbial community structure and microbial C:P ratios were more affected by tillage and depth than fertilisation (Shi et al., 2012); in tropical forests impacted by N-deposition, an old-growth forest showed increased microbial biomass and increased fungal PLFA's following P fertilisation, although there were no such effects in disturbed forests (Liu et al., 2012); and in a grazed grassland P additions reduced the fungal: bacterial ratio and led to a shift in microbial community structure (Parfitt et al., 2010). The conclusion that microbial processes dominate P dynamics in grassland soils (Bünemann et al., 2012) and that phosphate is an important driver of bacterial community structure in a range of soil types (Kuramae et al., 2012), also indicate a likely shift in microbial community structure. Effects of P fertilisation in grassland soils are not just limited to the microbial community as there are also effects on the soil food web, notably nematode community structure (Parfitt et al., 2010), nematode abundance and biomass (Vonk and Mulder, 2013) and the soil biomass spectrum (Mulder and Elser, 2009), with more large bodied fauna if P is not limiting.

Based on previous results from this long-term Irish P fertilisation trial (Culleton et al., 2002), with documented changes in plant community structure (King-Salter, 2008), the proportion of P-solubilising bacteria (Tan et al., 2013) and differential bacterial nutrient limitation (Griffiths et al., 2012), we hypothesised that there would be a shift in soil microbial community and food web structure in response to altered P fertilisation of this grazed grassland.

2. Material and methods

2.1. Site description and experimental design

The field site was established on a humic gleysol with a sandy loam texture at Johnstown Castle, county Wexford, Ireland (Culleton et al., 2002; King-Salter, 2008), with an annual average precipitation of 940 mm and mean air temperature of 10.2 °C from April, 2009 to April, 2011 (the duration of the described observations). At the start of the trial, in 1968, the site was ploughed and sown with *Lolium perenne*. Phosphorus (calcium superphosphate) was applied annually at 0 (P0), 15 (P15) and 30 (P30) kg P ha^{-1} to each of 12, 0.45 ha, replicate plots (36 plots in total). Nitrogen (ammonium nitrate, 240 kg N ha^{-1}) and potassium (potassium chloride, 20 kg K ha^{-1}) were also applied annually to all plots, P and K in spring and N during the growing season. Six plots of each P treatment were grazed at a low stocking rate (2200 kg beef cattle ha^{-1}) and the remaining six plots per P treatment stocked at a high rate (3300 kg beef cattle ha^{-1}). Each of the six plots was grazed in rotation according to sward height. In 1999 P application and stocking rates were altered, such that all plots now had the same stocking rate (3300 kg ha^{-1}) but of the former low stocking rate plots: P0 now received 30 kg P ha^{-1} yr^{-1} (P0-30); P15 received 5 kg P ha^{-1} yr^{-1} (P15-5); and P30 received 0 kg P ha^{-1} yr^{-1} (P30-0). Thus, since 1999 there were six replicate plots of six P treatments: P0; P0-30; P15; P15-5; P30; and P30-0.

2.2. Soil samples collection and preparation

Soil samples were taken in Spring 2009 (May) approximately one month after P and K fertilisation, Autumn 2010 (October) and Spring 2011 (April) prior to fertilisation. Thirty cores were taken in a stratified random design using an auger to a depth of 10 cm from each plot and mixed to give a composite sample from each plot. Prior to nematode extraction and soil microbial analysis, all fresh samples were stored at 4 °C. Moisture content of fresh soil was determined by drying at 105 °C for 12 h, while samples for nutrient analysis were dried at 40 °C for 24 h. In 2010, 2011 but not 2009, ca. 50 g of the composite soils was sieved (2-mm mesh) immediately after sampling and were stored in 10 g aliquots at −80 °C in plastic bags for phospholipid fatty acid (PFLA) analysis.

2.3. Soil nutrient analysis

Soil total P followed British Standards ISO 11466:1995, by extraction in Aqua Regia (3:1 hydrochloric acid and nitric acid) and determination by ICP (Olson and Sommers, 1982).

Morgan's P was determined using Morgan's extraction solution (10% sodium acetate in 13% acetic acid buffered at pH 4.8) (Peech and English, 1944; Byrne, 1979) and determined colourimetrically, using the reaction between P and ammonium molybdate (Murphy and Riley, 1962). pH was measured in deionised water (Thomas, 1996) and recorded with a Thermo Orion 420 pH meter.

2.4. Soil microbial properties

Soil from each composite sample was sieved through a 2-mm diameter mesh and stored at 4 °C prior to analysis. Soil microbial biomass C (MBC), N (MBN) and P (MBP) were determined by chloroform-fumigation extraction (Joergensen, 1995). For C and N, fumigated and non-fumigated portions of moist soil were extracted with 0.5 M K_2SO_4 (Jenkinson et al., 1988; Sparling et al., 2000). Total organic carbon (TOC) and total organic N (TON) were measured using a Shimadzu TOC-VCPH and a Shimadzu TNM-1 analyser respectively (Shimadzu Scientific Instruments, USA). Fumigated and non-fumigated P extractable in 0.5 M $NaHCO_3$ (Brookes et al., 1985) was measured by a modified ammonium molybdate–ascorbic method (Murphy and Riley, 1962). $NaHCO_3$ extractable P from the non-fumigated soil is subsequently referred to as Olsen's P. The *k*-factors used for converting extractable C, N and P flush to microbial C, N and P were 0.41 (Sparling et al., 2000), 0.45 (Jenkinson et al., 1988) and 0.40 (Brookes et al., 1985).

Subsamples of 10 g soil from each sample were freeze-dried for phospholipid fatty acid (PLFA) analysis (autumn 2010 and spring 2011) (Bossio and Scow, 1998). A total of 26 different PLFAs were detected and identified. The biomass of bacteria was determined using the combined weights of fatty acids i15:0, a15:0, 15:0, i16:0, i17:0, cy17:0, 17:0 and cy19:0. Fungal PLFA was determined as the sum of 18:2ω6c, 18:1ω9c and 18:1ω9t (Frostegård and Bååth, 1996; Bossio and Scow, 1998; Mikola and Setälä, 1998).

2.5. Soil nematodes

Nematodes were extracted from 100 g composite samples using an Oostenbrink elutriation – Baermann funnel extraction over 48 h (Verschoor and de Goede, 2000). Nematodes in the resulting suspension were killed at 65 °C for 4 min, preserved by 0.4% formaldehyde, and the first 150 individual identified microscopically according to Bongers and Yeates (1999). To define the nematode

Please cite this article in press as: Chen, X., et al., Microbial and microfaunal communities in phosphorus limited grazed grassland change composition but maintain homoeostatic nutrient stoichiometry, Soil Biology & Biochemistry (2014), http://dx.doi.org/10.1016/j.soilbio.2014.03.024