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Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil

J.C. Aciego Pietri [a], P.C. Brookes [b,*]

[a] Agriculture Faculty, Central University of Venezuela, Maracay, Aragua State, Ap. 4579, Venezuela
[b] Agriculture and the Environment Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

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ABSTRACT

Our aim was to determine whether the smaller biomasses generally found in low pH compared to high pH arable soils under similar management are due principally to the decreased inputs of substrate or whether some factor(s) associated with pH are also important. This was tested in a soil incubation experiment using wheat straw as substrate and soils of different pHs (8.09, 6.61, 4.65 and 4.17). Microbial biomass ninhydrin-N, and microbial community structure evaluated by phospholipid fatty acids (PLFAs), were measured at 0 (control soil only), 5, 25 and 50 days and CO_2 evolution up to 100 days. Straw addition increased biomass ninhydrin-N, CO_2 evolution and total PLFA concentrations at all soil pH values. The positive effect of straw addition on biomass ninhydrin-N was less in soils of pH 4.17 and 4.65. Similarly total PLFA concentrations were smallest at the lowest pH. This indicated that there is a direct pH effect as well as effects related to different substrate availabilities on microbial biomass and community structure. In the control soils, the fatty acids 16:1ω5, 16:1ω7c, 18:1ω7c&9t and i17:0 had significant and positive linear relationships with soil pH. In contrast, the fatty acids i15:0, a15:0, i16:0 and br17:0, 16:02OH, 18:2ω6,9, 17:0, 19:0, 17:0c9,10 and 19:0c9,10 were greatest in control soils at the lowest pHs. In soils given straw, the fatty acids 16:1ω5, 16:1ω7c, 15:0 and 18:0 had significant and positive linear relationships with pH, but the concentration of the monounsaturated 18:1ω9 PLFA decreased at the highest pHs. The PLFA profiles indicative of Gram-positive bacteria were more abundant than Gram-negative ones at the lowest pH in control soils, but in soils given straw these trends were reversed. In contrast, straw addition changed the microbial community structures least at pH 6.61. The ratio: [fungal PLFA 18:2w6,9]/[total PLFAs indicative of bacteria] indicated that fungal PLFAs were more dominant in the microbial communities of the lowest pH soil. In summary, this work shows that soil pH has marked effects on microbial biomass, community structure, and response to substrate addition.

1. Introduction

The effects of soil pH on specific microorganisms, or on the whole microbial biomass, microbial activity and, more recently microbial community structure, have previously been investigated (Anderson, 1998; Bååth and Anderson, 2003). Most studies on the effects of soil pH on microbial community structure have focused on forest soils (e.g., Frostegård et al., 1993a; Bååth et al., 1995; Blagodatskaya and Anderson, 1998; Pennanen, 2001; Bååth and Anderson, 2003). These different studies have demonstrated that soil pH and substrate availability are both important factors in determining the microbial community structure. For example, Frostegård et al. (1993a) monitored shifts in the microbial communities in soils from two different coniferous forests treated with lime or wood-ash. Five to six years later, changes in PLFA patterns indicated that the increase in pH caused a shift in the bacterial community to more Gram-negative and fewer Gram-positive bacteria, while the amount of fungi was unaffected. Bardgett et al. (1996) considered that fungal growth was influenced more by the input of organic substrate than by soil pH. They worked with four grassland soils from a long-term experiment but with a pH range possibly not wide enough to draw general conclusions (pH 4.5–5.4). Blagodatskaya and Anderson (1998) studied 40 forest soils with a much wider pH range (pH 3–6) and reported that, besides pH as the major controlling factor, substrate quality (as reflected by forest type) also influenced the fungal–bacterial ratio. In addition, a pH and substrate interaction occurred where the magnitude of the substrate effect was dependent on the prevailing pH.

Here, we report results from the Hoosfield Acid Strip, a site adjacent to the long-term (or classical) Hoosfield arable field

* Corresponding author. Tel.: +44 1582763133; fax: +44 1582760981.
E-mail address: philip.brookes@bbsrc.ac.uk (P.C. Brookes).

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experiments at Rothamsted Research, UK. Soil from the Hoosfield acid strip has a wide range of pH values (from about 8.3–3.7) which form an extremely uniform pH gradient, as a result of a single large application of chalk during the 19th century. It has not received any other amendment including chemical or organic fertilizer since then (Aciego Pietri and Brookes, 2008). Our aims were to test whether changes in microbial biomass, microbial activity and microbial community structure are directly caused by changing pH, or due to changing substrate input, or both. This is a difficult question to answer directly. Generally, the effects of decreasing pH and decreasing substrate inputs are confounded as plant inputs, especially in arable situations, often decline sharply below about pH 5. We attempted to separate these effects in soils of decreasing pH taken along the pH gradient of the Hoosfield Acid Strip. Accordingly the soils, at pHs 8.09, 6.61, 4.65 and 4.17, were incubated under laboratory conditions with or without added wheat straw. Microbial biomass ninhydrin-N, microbial activity as CO_2-C evolved and Phospholipid Fatty Acids (PLFAs) were determined. In this way, by supplying additional substrate to low pH soils, it was hoped that we could separate these two main effects.

2. Materials and methods

2.1. Sampling and preparation of soils

Soil sampling was carried out at four core positions with different pHs along the Hoosfield Acid Strip. Full details of the pH gradient and how it was formed, more than 100 years ago, are given by Aciego Pietri and Brookes (2008). At each sampling position, twenty cores, each five cm diameter (0–23 cm depth) were taken across the strip and bulked in the field. All the soil samples were sieved moist separately (<2 mm) and their dry matter contents determined (105 °C, 24 h).

2.2. Soil treatments

The soils, sampled as described above, had pHs of 8.09, 6.61, 4.65 and 4.17, respectively. They were amended or not with wheat straw (oven-dried and milled to 1–2 mm before use) to give the following treatments: (1) soil alone (control soils), and (2) soils given wheat straw and fertilizer (termed 'straw' throughout). The straw amendment (1%, weight/weight) provided 4600 μg C g^{-1} soil. Also, 266 μg N g^{-1} and 100 μg P g^{-1} soil were added as aqueous NH_4NO_3 and KH_2PO_4 in the volume of water necessary to adjust to 40% WHC (or 50% WHC for the soils given straw, to compensate for the increase in dry matter). The inorganic N was added to adjust the C/N ratio of the added substrate to approximately 20 (excluding the N already contained in the straw) and KH_2PO_4 was added to avoid any possibility of P deficiency. Approximately 150 g portions of each treated and control soil were placed in plastic bags as individual replicates and arranged in a randomized block design. Each of three replicates were placed in separate air-tight metal drums (70 × 40 cm) and incubated for 50 days at 25 °C in the dark, with several 100 ml jars containing soda lime (to absorb CO_2 evolved) and several open bottles containing distilled water (to give 100% humidity). The drums were opened every few days, as is our standard practice, to ensure adequate aeration.

2.3. Soil chemical analyses

Soil pH was measured at a soil:water ratio of 1:2.5 (weight/weight). Air-dry soil (10 g, <2 mm) and 25 ml of deionised water were shaken together for 1 min, left to settle for 30 min, repeating this procedure once more, then pH determined with a pH electrode. Full details of soil chemical analyses are given by Aciego Pietri and Brookes (2008).

2.4. Soil microbial analyses

2.4.1. Microbial biomass

K_2SO_4-extractable ninhydrin-nitrogen was measured by the method of Amato and Ladd (1988) as modified by Joergensen and Brookes (1990). Soil microbial biomass ninhydrin nitrogen (biomass ninhydrin-N; B_{NIN}) was calculated from: B_{NIN} = [(ninhydrin-N extracted from fumigated soil) minus (ninhydrin-N extracted from non-fumigated soil)] and expressed as μg ninhydrin-N g^{-1} soil.

2.4.2. Microbial activity

Microbial activity was assessed by measuring carbon evolved as CO_2-C. Each moist soil portion (50 g on an oven-dry soil basis) was put into a glass jar (100 ml) which was then placed in a 1 l brown glass jar with 25 ml 1 M NaOH in a vial and 10 ml free water. The jars were sealed with a rubber bung. Three blank replicate jars without soil were also prepared. After the required period of incubation, each NaOH sample (5 ml) was mixed with water (10 ml) and auto-titrated from pH 8.3 to 3.7 with standard 0.5 M HCl (Tinsley et al., 1951). Cumulative amounts and rates of CO_2-C evolved were expressed as μg CO_2-C g^{-1} soil and μg CO_2-C g^{-1} soil d^{-1} respectively.

2.4.3. Microbial community structure

Microbial community structure was determined by PLFA analysis, using separate portions of the soils from the incubation experiment which was stored at −15 °C in glass bottles until analysis. The PLFAs were extracted from these soils by a modified Bligh and Dyer method (White et al., 1979). The total PLFA concentration (expressed as ng g^{-1} soil) was calculated as the sum of all identifiable and unidentifiable PLFAs. The production of methyl ester fatty acids from phospholipids was done by a methylation procedure. Briefly, the dried phospholipids fractions were re-dissolved in methanol:toluene (1:1; v/v), a solution of 0.2 M KOH–methanol was added and heated at 37–40 °C for 15 min, shaking periodically and left to cool to room temperature. Then, 0.2 M acetic acid was added to adjust the pH to 7. Chloroform and water were added, mixed on a vortex mixer then allowed to stand for 30 min at 4 °C as two phases. Finally, the lower chloroform phase was transferred to a clean tube and the chloroform evaporated under a stream of N_2. The vials were then stored at −15 °C until analysis.

The PLFA profiles were determined by gas chromatography (GC) using a Hewlett Packard GC series 6890 with flame ionisation detectors by injection of aliquots (1 μl) of extracts. A non-polar column (95% dimethyl-5% diphenyl polysiloxane, length 30 m and i.d. 320 μm) was used to separate the PLFAs. Nitrogen was the carrier gas. The oven temperature started at 70 °C for 1 min, and increased to 150 °C at 20 °C min^{-1}, and then further increased to 280 °C at 5 °C min^{-1}. The total run time was 31.5 min. Each run was identified by comparing retention times with an external standard (BAME, 47080-U, Supelco, Poole, UK). Peak areas were quantified from peaks generated by blanks containing an internal standard (14:20H, 100 ng μl^{-1}, Sigma–Aldrich). Integration and analyses of peak data were done using the Hewlett Packard software ChemStation Data System 33988A. As the response of the Flame Ionisation Detector is proportional to molecular weight, the concentration of each individual PLFA was assumed to be proportional to its peak area and was calculated from: ng individual fatty acid g^{-1} soil = (P_{FAME} × ng Std)/(P_{ISTD} × dilution × W), where P_{FAME} and P_{ISTD} are the peak areas of each fatty acid methyl ester and a standard respectively, ng Std is the concentration of a standard