Soil without plants: the consequences for microorganisms and mesofauna

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Abstract

Plants provide the primary carbon source for soil communities, but there are few studies on the consequences of their absence over an extended period. It is unclear whether a reduction in the number of organisms associated with diminishing resources is associated with a reduction in their diversity. To investigate this, soil organic carbon (SOC), microbial and mesofaunal communities in the Highfield Ley-Arable Experiment at Rothamsted Research were compared. Samples were taken from an old grass sward, a section converted to arable rotation 60 years ago, and a section regularly tilled to maintain a bare fallow for the past 50 years. Microbial biomass is positively correlated with SOC regardless of the specific composition of the carbon, but the diversity of the community does not appear to be influenced by these parameters. In contrast, the abundance and diversity of the soil mesofauna are strongly influenced by recent plant inputs. On the fallow plot, the virtual elimination of fresh carbon inputs results in the degradation of SOC and reduction in microbial and mesofaunal numbers. However, the plot supports a species-rich and metabolically active bacterial community that is not significantly reduced in diversity compared to soil under arable cultivation or maintained as grass.

Key Words

Soil management, ley-arable rotation, community structure, diversity index

Introduction

Carbon inputs to soil from recent photosynthesis are of similar importance to decomposition of more recalcitrant organic matter in driving biological processes in soil: the plant community influences the community composition of the rhizosphere and soil biota, which in turn influence plant productivity (Wardle *et al.* 2004). Other than a few groups of chemoautrotrophic bacteria, the majority of soil organisms rely on fixed carbon from root exudation and other plant residues together with the excreta and remains of soil fauna and inputs from above-ground mega fauna. To establish the extent to which plant inputs affect below-ground communities, we investigated the long-term Highfield Ley-Arable Experiment at Rothamsted, where parts of a long-standing grass ley were ploughed in 1949 and managed subsequently by arable rotation. In addition, since 1959, one area has been maintained, by regular tillage, as bare-fallow. The composition of organic inputs is determined by the plants that are present, so a mixed grass sward is likely to provide a more complex substrate than a wheat monoculture. The amount of soil organic carbon (SOC) in soil is a balance between what plants supply to the soil through the roots and, in managed systems, crop removals, any returns of straw or stubble, and the decomposition of SOC already present (the 'native' pool). Previous work at this site comparing the arable soil with permanent grass has graphically illustrated the decline of SOC over time associated with conversion from grass to arable cultivation (Johnson *et al.,* 2009). We tested the hypothesis that the bare-fallow soil supports smaller and less diverse microbial and mesofaunal communities than soil from the more substrate rich arable and grass plots.

Methods

Site and sampling regime

Highfield soil is a silty loam over clay (Batcome series) - a Chromic Luvisol by FAO criteria. Prior to the summer harvest, we sampled: (i) soil continuously under arable crops in rotation (winter wheat at time of sampling) since 1949; (ii) soil from an adjacent area of unmanaged mixed grass sward unchanged for at least 200 years: (iii) soil from an adjacent bare-fallow strip which has been maintained crop- and weed-free since 1959 by tillage whenever weed growth was apparent (minimum three times a year). Arable crops were managed as normal farming practice, receiving NPK fertilizer, fungicides and insecticides as required. Lime had previously been applied to maintain the pH at c 6.0, although pH had been allowed to decline in the decade prior to this sampling; fertilizer had last been applied to the arable crop two months prior to sampling. An earlier investigation (Watts and Dexter, 1997) found that the soil under permanent grass contains much more organic carbon than that under arable and bare-fallow, and that soil physical properties follow the same trend: aggregate stability and water retention were found to be greatest under grass and least under bare-fallow. The sampling strategy, SOC)and its sub-fractions, soil pH, the total microbial biomass and its diversity, the abundance of heterotrophic bacteria and the sub-group pseudomonads, soil mesofauna (collembola and mites belonging to the Oribatei or other groups), phospholipid fatty acids (PLFAs), Biolog™ substrate utilisation by active bacteria, 16S rRNA and DNA abundance and diversity using DGGE were measured as described by Hirsch *et al.* (2009). The MultiVariate Statistical Package MVSP version 3.13d (Kovach Computing Services) was used to estimate Shannon Diversity Indices *H´* from qualitative and quantitative data, PLFA HPLC traces, band position and intensity on DGGE gels, and for Principle Component Analysis (PCA) of this data.

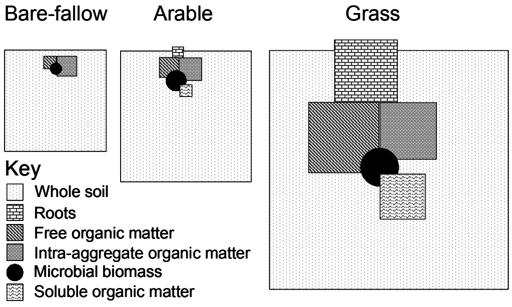
Soil organic carbon fractionation

Soil fractions were separated by their density to reflect the *in-situ* position of the materials within the soil matrix (Sohi *et al.* 2001). The fractions include free organic carbon – discrete organic particles of non-specific size, located outside stable aggregates, and intra-aggregate organic matter – discrete organic particles released from their enclosure within stable aggregates by ultrasonic disruption. The former fraction is assumed to be more available and the latter more recalcitrant to mineralization by the soil microbiota. The sum of these fractions was subtracted from the total SOC to estimate the soluble fraction that includes root exudates and microbial metabolites.

Results

Total soil organic carbon

Arable and old permanent grass soils had similar pH (5.5); the bare-fallow soil a slightly lower (pH 5.1). A higher carbon (C) content of the grass soil (5.39 %) compared to the arable (1.62 % C) and bare-fallow soil (0.97 % C) was consistent with the previous finding that it had a lower aggregate density (Watts and Dexter 1997). The C: N ratio increased bare-fallow<arable<grass, probably reflecting the higher proportion of fresh roots in the grass soil, with no roots detected in the bare-fallow. Soil under grass contained more than three times the SOC concentration than the arable soil in the 0-10 cm layer, i.e. significantly more potential substrate for soil biota; the arable soil contained two-thirds more SOC than the bare-fallow soil. In the arable soil, C inputs are mixed through the top 23 cm by annual tillage; in the bare-fallow soil the minimal input from occasional weeds is similarly homogenised by more frequent tillage. Thus, after almost 50 years without significant plant input, the bare-fallow soil contains ~ 30 t C ha^{-1} as recalcitrant SOC, <8% of that in the original grassland soil. Figure 1 illustrates graphically the relative differences in SOC and the proportions of different fractions, most obviously the relative decline in free SOC and corresponding increase in the more recalcitrant intra-aggregate C fraction when grass, arable and bare-fallow soils are compared in that order, but microbial biomass C remained a fairly constant proportion of the total SOC. The biomass-specific respiration was higher, and similar, in grass compared to bare-fallow and arable soils.

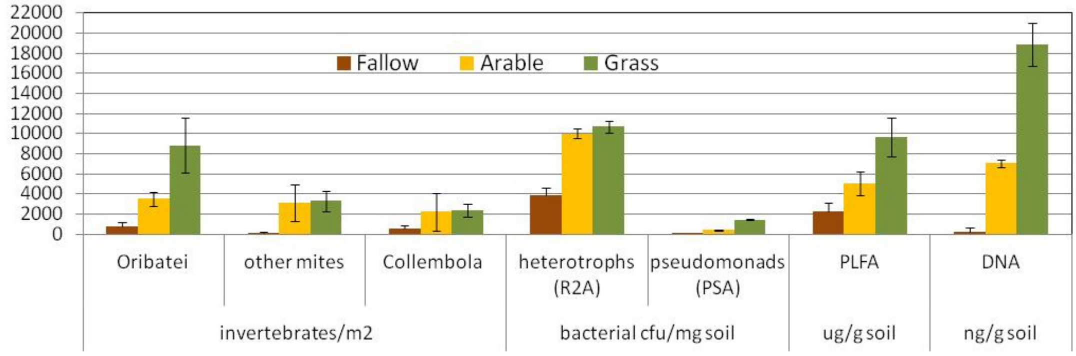


The area of the large squares represents the relative amount of total organic C in each soil; superimposed on these, the areas of the smaller shapes represent the relative amount of each discrete organic C fraction, assuming that the soil aggregate density is similar to the bulk density and that the C content of root dry matter is 40 % (Robinson, 2001).

Figure 1. Distribution of organic carbon amongst different components of the three soils.

Soil biomass and the relative abundance of biota

Soil biomass analyses (C, respiration, PLFA and DNA yields), numbers of culturable bacteria, and soil invertebrates all reflected the SOC, which provides substrate for the soil biota. The total number of mites showed a similar trend to SOC and biomass, with most in the grass and fewest in the bare-fallow soil (Figure 2). This was clearly demonstrated in the subgroup Oribitei. The other mites and the collembola showed a similar trend but mite numbers were not significantly different between arable and grass soils, and collembola numbers were significantly different only between the grass and bare-fallow soils.

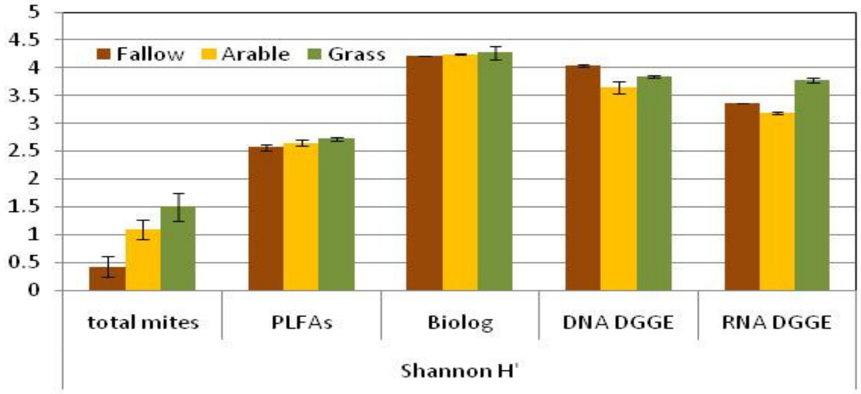


Note differences in scale of each measurement to allow results to be displayed on the same numerical axis. Standard errors of the means are shown.

Figure 2. Relative abundance of mesofauna, culturable bacteria and microbial biomarkers in soils

Estimates of diversity

The Shannon diversity index *H´* of mites was significantly different in the three soils, following the order grass>arable>bare-fallow. However, this marked trend which followed the differences in SOC and biomass was not seen in the other communities (Figure 3). PLFA analysis, which indicates overall soil microbial diversity (bacteria and fungi) followed the same trend, albeit with very small differences, but the Biolog *H´* was not significantly different, possibly because this method is indicative only of the small sub-population that can grow in the different Biolog substrates (Degens and Harris, 1997; Nannipieri *et al.,* 2003). A further departure from the grass>arable>bare-fallow trend was observed with the DNA and RNA-based methods. More individual 16S DGGE bands arising from DNA, indicating the relative abundance and number of bacterial species, were detected in the bare-fallow soil (61) than in the grass (50) or arable (44) soils. The analysis of rRNA molecules (RNA derived from ribosomes in recently active cells, indicating metabolically active species) reversed this trend, with 31 bands seen in bare-fallow compared with 50 in grass, although arable again had fewest (26). The relative intensity of each band, in addition to the abundance of bands, was used to estimate *H´*, which reflects species abundance (Figure 3).



PCAs based on the relative positions and intensities of bands on DGGE gels differentiated the three soils. The metabolically active species represented by bands from RNA molecules appeared similar to the total species composition represented by DNA for grass, but were distinct and different for the other two soils indicating that each soil has a different active subset of bacteria. Standard errors of means are shown.

Figure 3. Shannon diversity indices, *H'*

Discussion

Total soil organic carbon

The greater abundance of soil mesofauna under grass compared to bare-fallow and arable soils is associated with the availability of plant material, exemplified by the abundance of roots under grass and their absence in the bare-fallow soil. Similarly the larger microbial community in the grass soil may be because substrates are more readily available to microorganisms as well as more plentiful than under arable or bare-fallow soil. By virtue of being in an accessible location within the mineral soil matrix (outside stable soil aggregates), free organic C may reflect the most readily available portion of native SOC. Its presence in the bare-fallow soil is somewhat surprising, given the long-term absence of crops and the rigorous control of weeds and it may comprise relict material rendered inaccessible by the absence of free nutrients. A previous thermo-chemical analysis of the fractions from the same plots indicated that free organic matter in the bare-fallow resembled, chemically, intra-aggregate SOC from typical soils (Lopez-Capel *et al.,* 2005) and the dramatic reduction in SOC included changes in the relative amounts of available C in fractions that were free or associated with aggregates, compared the original permanent grassland. The soil that had been reverted to arable cultivation showed a less dramatic but significant reduction in these factors.

Soil biomass and the relative abundance of biota

Biomass measures (C, respiration, total PLFA, total DNA), the number of culturable bacteria, and soil invertebrates all reflected the trend grass>arable>bare-fallow in SOC. The transient weed growth in bare-fallow soil appears to provide only limited resources that support a much-reduced mesofaunal community, including the fungal-feeding collembola and the mites which may be fungivorous, herbivorous or predatory. The pseudomonads are Gram-negative proteobacteria known to flourish in the rhizosphere (Lugtenberg *et al.* 2001), so their paucity in the bare-fallow soil is unsurprising.

Conclusions

Despite differences in community structure there was little to distinguish *H′* in the three soils, whether based on the presence and abundance of individual PLFAs, substrate utilization by active bacteria, or the relative abundance and diversity of total and active bacterial species based on 16S rRNA and DNA. However, the distinct structure of microbial communities in the three soils (in contrast to the similar diversity indicated by *H′*), in particular in the bare-fallow soil that is starved of plant inputs and tilled regularly, demonstrates that the variety of niches available for bacteria. The small inputs of C from soil autotrophs and occasional weeds are sufficient to maintain a functionally diverse community for decades. Results support previous reports that soils under arable cultivation support less diverse microbial communities than those under unimproved pasture (Degens *et al.,* 2000; Garbeva *et al.,* 2007). Additionally, the results may indicate a significant change in community structure in the absence of a dominant soil biota (in contrast to the grass and arable soils) to one that thrives on the more chemically recalcitrant forms of free C that are present in this starved soil (resembling the intra-aggregate fraction in the other soils), revealing a more specialised albeit genetically diverse community adapted to the recalcitrant intra-aggregate C.

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