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Microbial mechanisms of carbon and nitrogen acquisition in contrasting urban soils

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ABSTRACT

Urban soils play an essential role in delivering ecosystem services due to soil microbial functions but there is limited evidence of the role of urban soils in the global carbon cycle. Inorganic nitrogen (N) reduces microbial respiration of soil organic matter (SOM) in pristine and managed forest soils but there is less evidence available on the extent to which this occurs in contrasting urban soils. This study examined the ephemeral effect of inorganic N and SOM (woodland versus grassland urban soil) on microbial functions represented by extracellular enzyme activities and microbial respiration of added substrates of contrasting quality. It was hypothesized that inorganic N stimulates extracellular enzyme activities and microbial respiratory responses to the addition of substrates varying in SOM quantity or quality. Results showed significantly higher SOM content, DOC and dissolved phenolic compounds in the woodland compared to grassland soil. In the woodland soil only, N addition increased β-glucosidase and *N*-acetyl-glucosaminidase enzyme activities and decreased microbial respiration responses to substrates. This suggests a microbial requirement for C acquisition dependent on N availability that reduced overflow respiration of the microbial community due to the composition of the woodland SOM pool. In conclusion, urban soils that contrast in vegetation types and hence OM content will likely differ mechanistically in response to increased N deposition and climate change altering their potential ability to store soil C in the future.

1. Introduction

In urban ecosystems, soils play an essential role in delivering ecosystem services via the soil microbial community. However, the microbial community is not generally taken into consideration in the sustainable development of urban ecosystems [1]. Urban land-use change has been identified as one of the major components of environmental change because of its effects on climate, water, biodiversity, carbon (C), and nutrients across large areas of the globe [2]. Despite the growing body of literature [3] many aspects of urban ecosystem services have not been studied conclusively and empirical evidence is still scarce [4].

There is considerable interest in understanding the biological processes that determine C storage in soils in order to better understand mechanisms to limit anthropogenic climate change [5]. Soil organic matter (SOM) provides C to a range of soil organisms, being utilised as the basis for a range of organic molecules and is essential for providing the energy at the base of food webs [6,7]. Decomposition of SOM and ultimately carbon dioxide (CO_2) release depend on the combined response of extracellular and intracellular (microbial), enzymatically mediated reactions [8]. Extracellular enzymes catalyze the initial hydrolysis of a variety of complex polysaccharides in soil to simple monomers that can be transported actively and passively into microbial cells and catabolized by intracellular enzymes producing CO_2 [8]. The quantity and quality of SOM is known to affect soil enzyme activities, microbial respiration, and microbial biomass, and these in turn will impact on soil C storage via greenhouse gas production [5,9,10]. Edmondson et al. [11] have shown that organic C storage may be significantly greater in urban soil than in regional agricultural land at equivalent depths. However, the microbial mechanisms explaining C storage in contrasting urban soils are not fully understood.

The nitrogen (N) cycle has been perturbed since post-industrial times through enhanced reactive N in the form of anthropogenic sources such as fossil fuel burning and agricultural fertilizers with implications for microbial functions and C dynamics within urban soils [12–14]. It has been argued that N deposition is a fundamental driver of increased C sequestration in forest ecosystems, significantly affecting the C balance of temperate and boreal forests [15]. Janssens et al. [16] proposed that the mechanism for this increased C sequestration has occurred from N stimulating woody biomass at the expense of below

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ground C allocation. As a result, two simultaneous effects occur: (1) an increase of recalcitrant sources of C into forest soil ecosystems as the additional woody biomass enters soil C pools as leaf litter and (2) decreasing labile C through root exudation. This results in a decrease of microbial biomass and respiration that increase soil C storage.

Kleber [17] and Schmidt et al. [18] recently discussed in detail how the molecular structure (recalcitrance) of SOM alone does not control SOM stability. Labile organic carbon has been defined [19] as being both chemically degradable and physically accessible by soil microbes whilst availability of SOC has been defined [20] as the biochemical recalcitrance of organic compounds, that is, their susceptibility to enzymic degradation with further uptake of reactive products by soil microorganisms. Soil microbial communities require C and nutrients to synthesize extracellular enzymes to breakdown recalcitrant SOM [21]. Therefore, nutrients such as N can become a limiting factor for growth of microorganisms reliant on recalcitrant sources of SOM [22]. However, the production of extracellular enzymes by microbes represents an energetic cost [14,23] and enzymes will only be synthesized when available nutrients in simple forms are scarce resulting in the utilisation of more complex and stable forms [14]. In C rich soils, N availability can limit extracellular enzyme synthesis that is required to breakdown complex C polymers to simple forms that subsequently enhance microbial activity and growth [24]. However, N addition to soil has been found to decrease microbial decomposition and respiration [25,26] especially for the SOM pools that cycle slowly [27,28]. Spohn [29] suggested three mechanisms to explain these changes: (i) microorganisms 'mine' litter for N, burning readily available (or labile) C in order to gain energy to acquire N from more recalcitrant forms of SOM containing a higher C/N ratio [29–31] – microbial C limitation will be regulated by the return on investment in extracellular enzymes that depends on the availability of N [14]; (ii) microorganisms uncouple respiration from energy production and only respire easily available C to dispose of it via 'overflow respiration' to maintain the stoichiometric ratio of C/N [32] – when growing on N-poor substrate, microorganisms do not have enough N to build up as much biomass as the C concentration would allow due to stoichiometry [29]; and (iii) the activity of oxidative enzymes involved in the degradation of aromatic compounds decreases with increasing N concentration [33] suggesting that lignin degradation is a mechanism of N acquisition by mining. As urban environments are characterized by high levels of N deposition [13], this raises the question of whether direct N deposition to soil (i.e. mineral versus organic N), leading to decreased litter C/N ratios, might control C storage by driving microbial mechanisms of C and N acquisition in specific urban soils.

This study examined the ephemeral effect of inorganic N addition on extracellular enzyme activities and microbial respiration by adding substrates of varying quality in two urban soils (woodland and grassland) located in close proximity and characterized by contrasting SOM pools (Table 1). Woodland soils typically have sparse understorey vegetation and C accumulates from dead leaf litter and detritus that are decomposed to produce humified recalcitrant SOM [34]. By contrast, grassland plant communities have been shown to positively affect the supply of root exudates suggesting rhizopheric microbes dominate with limited requirement for the production of C degrading enzymes [35]. Thus, woodland soils generally consist of higher amounts of SOM with recalcitrant C (i.e. aromatic phenolics) whilst grassland has a greater availability of faster cycling labile C reflecting differences in SOM quantity and quality. We compared the activities of the extracellular enzymes β-glucosidase, *N*-acetyl-glucosaminidase and phenol oxidase, as these enzymes are involved in the decomposition of cellulose (a major type of complex C compounds in soil), chitin (a significant fraction of humus-bound N in soil) and polyphenolic substances (slowly decomposing complex aromatic compounds). The substrates chosen for microbial respiration using the MicroResp™ method were carbohydrates/complex organic polymers (D-glucose, D-arabinose, D-galactose, fructose, D-trehalose, sucrose, cellulose, lignin) or amino/carboxylic acids (L-arginine, L-alanine, glycine, γ-aminobutyic acid, L-malic acid, citric acid) representing a range of important exudates and labile or recalcitrant organic substrates in soil to investigate the microbial respiration response and substrate preference by the microbial community. It was hypothesized that inorganic N would stimulate extracellular enzyme activities and microbial respiration of added labile C substrates but that this response would be dependent on soil characteristics of the grass and woodland soil (i.e. SOM content, phenolics).

Table 1
Physicochemical characteristics of the grass and woodland soils. Data are mean (standard error); ns = not significant.

Soil characteristic	Grassland	Woodland	*P* value
pH	5.49 (0.066)	5.32 (0.066)	ns
Gravimetric water content (%)	64.7 (0.406)	62.1 (0.651)	ns
Ammonium (mg L^{-1})	1.20 (0.064)	1.26 (0.094)	ns
Nitrate (mg L^{-1})	2.07 (0.760)	4.30 (1.030)	ns
Total inorganic N (mg L^{-1})	3.32 (0.782)	5.60 (1.100)	ns
SOM (%)	17.1 (0.096)	24.4 (0.391)	0.001
Phenolics (mg L^{-1})	5.12 (1.120)	9.28 (0.719)	0.01
Absorbance (254 nm)	0.03 (0.006)	0.29 (0.012)	0.001

2. Materials and methods

2.1. Site selection and soil sampling

The study area, Stoke Park Estate, is an urban, public area, located in close proximity to the M32 motorway in the north of Bristol, UK (Fig. 1). Soil was collected from a seasonally wet semi-natural grassland pasture (*Arrhenatherum elatius*) measuring approximately 16 ha and ancient lowland mixed broadleaf woodland (*Quercus robur, Fraxinus excelsior, Fagus sylvatica*, *Aesculus hippocastanum*) measuring approximately 8 ha (UK grid reference: 51.494827, −2.553233). The grassland area was likely originally part of the woodland in the past as can be seen from the regular shape of the grassland. These two habitats were chosen as they are located in close proximity that minimizes variability in confounding factors such as weather and physicochemistry. Despite the differences in aboveground vegetation and hence SOM that was of interest as a factor, the sites were also chosen as the soil types are both defined as the Denchworth vegetation type (712 b) (Stagni-Vertic Cambisol under FAO classification). This soil type is defined as slowly permeable, seasonally waterlogged, clayey soils with similar fine loamy over clayey soils [36–38].

On the 6th of November 2014, surface leaf litter, detritus and/or grass leaves/roots were removed from a representative area in each habitat measuring approximately 10 m^2. Soil samples were pooled from 4 spatial replicates to 15 cm below the O horizon. These sites were chosen due to the likelihood of differences in SOM quantity (i.e. loss on ignition) and quality (i.e. phenolics, SOM/inorganic N) appropriate for this laboratory study rather than as a comparison of habitat types. Sixteen samples of 220 g of soil (wet weight) were homogenised through a 1 mm sieve and placed in 16 × 1 L containers (8 × woodland and 8 × grassland soils). The soils were prepared within 24 h and incubated at field temperature (13 °C). Within each vegetation type, at week 1 and week 3, four randomly selected replicates were treated with 30 ml of deionised water and the remaining four treated with 30 ml of 0.125 M NH_4NO_3. This amount was chosen according to DEFRA fertilizer guideline application rates [39]. The applications of N were split in two applications to prevent osmotic shock and applied uniformly across the soil surface. The experimental design was fully factorial with SOM (site) x NH_4NO_3 treatment allowing for interaction effects.

Gravimetric water content was adjusted to 65% in all jars until week 4 when moisture was reduced to 44% for the MicroResp™ assay [40]. Soil moisture was determined every 72 h for moisture loss by weight and moisture was replaced by equivalent amounts of deionised water.