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Comparison of fertility and seasonal effects on grassland microbial communities

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ABSTRACT

The activity of saprotrophic fungi and bacteria, and the balance between them, can affect decomposition. Arbuscular mycorrhizal (AM) fungi are also important for the nutrient and energy transfer in soil. Microbial community composition and activity are believed to have seasonal patterns, and are known to be highly influenced by environmental factors such as pH and nutrient conditions. To evaluate the importance of season for the variation in microbial decomposer community in a context of well-known environmental factor variation, we studied microbial growth, biomass and community structure along a fertility gradient (pH 5.9–8.1; NH_4-N 3–19 μg g^{-1} soil, f.w.) in a sandy grassland during one year. The microbial community structure (phospholipid fatty acid (PLFA) composition) and biomass (PLFA and neutral lipid fatty acid (NLFA) signatures) as well as fungal (acetate incorporation in ergosterol) and bacterial (leucine incorporation) growth rates were investigated at eight seasonal time points during one year. The environmental factors pH and NH_4 concentrations explained a larger share of the variation in the microbial community structure. Together they explained 37% of the variation, while season (proxied by temperature) only explained 6% of the variation in PLFA composition. Bacterial and fungal biomass were both highest in early spring, while AM fungal biomass peaked in early summer. Bacterial growth rate, on the other hand, was highest during the autumn, while fungal growth rate showed no clear seasonal pattern. In conclusion, the influence of seasonal variation on microbial communities proved to be relatively small compared to that which could be assigned to pH and NH_4 in the studied ranges.

1. Introduction

Microorganisms in soil control carbon (C) storage and nutrient cycling (Schimel, 1995), and the relative contribution to decomposition by fungi and bacteria can be used to characterize the soil food webs (Moore et al., 2005; Neutel et al., 2007). The relative importance of bacteria and fungi has also been reported to vary over the year (Bardgett et al., 1999a; Lipson et al., 2002; Schadt et al., 2003; Habekost et al., 2008), with implications for the C cycle. An anticipated change in climate (IPCC, 2007), with warmer winters in the northern temperate areas, might alter the annual variation in microorganisms (Birgander et al., 2012). In grasslands, arbuscular mycorrhizal (AM) fungi can influence the C and nutrient cycles, as a potential recipient of a large part of the plant fixed C (Drigo et al., 2010), by constituting a significant part of the soil microbial biomass (Olsson et al., 1999) and by competing for soil nutrients with the microbial decomposer community (van der Heijden et al., 2008; Leigh et al., 2011).

In temperate ecosystems there is an extensive seasonal variation in factors such as temperature and nutrient availability (Waldrop and Firestone, 2006) that could translate to changes in microbial community composition. Activities generally decrease during winter due to the low temperature, although it has been shown that both plants and microbes can be active during the winter (O'Neill, 2000; Starr and Oberbauer, 2003; Cao et al., 2004; Steenberg Larsen et al., 2007; Lekberg et al., 2013). Pronounced seasonality in the microbial community has been reported both from studies in temperate (e.g. Bardgett et al., 1999a; Habekost et al., 2008; Regan et al., 2014) and arctic systems (e.g. Lipson et al., 2002; Schadt et al., 2003), but there appears to be a lack of systematic pattern from previous work. For instance, there have been suggestions that the total microbial biomass in temperate ecosystems can peak either in autumn (Habekost et al., 2008; Regan et al., 2014) or spring (Bardgett et al., 1999a). In arctic systems the highest microbial biomass has been found in the winter and the lowest in summer (Lipson et al., 1999; Schadt et al., 2003). The

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dominance of fungi of the soil microbial biomass has been reported to be higher in spring and autumn in temperate grassland (Bardgett et al., 1999a). In contrast, the proportion of fungal biomass compared to bacterial biomass was highest in the winter and decreased in the summer in alpine systems (Lipson et al., 2002).

The AM fungi are dependent on a symbiosis with plants, and their responses are thus likely to mirror those of plants. In line with this expectation, the intensity of the AM fungal root colonization has been shown to be linked with plant growth in tall grass prairie grassland (Bentivenga and Hetrick, 1992), low alpine meadow (Ruotsalainen et al., 2002), agricultural fields (Gavito and Varela, 1993) and costal sand dunes (Sigüenza et al., 1996). Consequently, AM fungal root colonization generally peaks in spring or summer when plant growth is at its maximum. One exception to this pattern was put forward by Lekberg et al. (2013) who did not find any seasonality in root colonization over one year in a sandy temperate grassland. However, NLFA 16:1ω5 in roots, an indicator for AM fungal storage structure did increase from spring to autumn.

As pointed out by Buckeridge et al. (2013) we cannot be certain if the high microbial biomass commonly found in spring is due to enhanced growth during winter, or a buildup during winter due to reduced predator activity during the winter (Preisser et al., 2006). Phospholipids are often assumed to be rapidly metabolized, and phospholipid fatty acid (PLFA) signatures are therefore thought to reflect active biomass (White et al., 1979), but these assumptions are currently questioned (de Vries et al., 2009; Frostegård et al., 2011). The contribution of dead organisms to PLFA signatures is a problem in particular when sampling during the winter, as degradation rates decrease at low temperatures (Ranneklev and Bååth, 2003). In a comparison between PLFA and neutral lipid fatty acid (NLFA) biomarkers, and microscopic counts, de Vries et al. (2009) found PLFA and NLFA signatures to be less responsive to decline compared to microscopic counts. Similarly, Rousk and Bååth (2007) showed that pronounced effects of plant material amendments on microbial growth rates did not always translate to detectable biomass changes, as determined with PLFA concentrations, even within weeks.

Sandy grasslands in southern Sweden are useful systems to study effects of abiotic factors on the microbial community. The habitat type is exposed to severe summer droughts and holds large variation in nutrient, disturbance and pH within short distances (Olsson et al., 2009; Mårtensson et al., 2012), factors that are also known to have a substantial influence on the microbial community composition (Rousk et al., 2009; Aliasgharzad et al., 2010; Rousk et al., 2011). By including a comprehensive gradient of fertility factors for each time point sampled over the year, we sought to compare and relate the well-recognized importance of soil fertility factors to that of seasonal variation on the soil microbial community. Some studies have investigated seasonal variation in microbial communities in temperate climates, but sampling intensity has often been low (four sampling points for example by Bardgett et al. (1999a) and Hamel et al. (2006), and as low as two sampling points by Habekost et al. (2008), Koranda et al. (2013) and Thoms and Gleixner (2013)). A previous study assessed the seasonal change in microbial PLFA concentrations along a fertility gradient created by nitrogen fertilization management (Bardgett et al., 1999a), but limited information of soil fertility factors made explicit comparison of seasonal effects to fertility influence difficult. This made the utilization of this second aspect (fertility, in addition to season) problematic to use as a positive control for known effects. The aim of our present study was to put the annual differences in microbial communities into a context with a substantial gradient of environmental factors (pH and fertility), at a high resolution, and thus to investigate and evaluate the impact of seasonality on the microbial community. We aimed to answer the following questions: (1) How does microbial biomass and growth vary during the year in a sandy temperate grassland? And if it does vary, (2) how does the annual variation relate to the variation along a fertility gradient?

2. Materials and methods

2.1. Study area and soil

A sandy calcareous semi-natural grassland in southern Sweden (N 55° 42′; E 14° 10′) was sampled between March 2010 and March 2011 (mean annual temperature 7 °C, precipitation 600 mm year^{-1}). The lime-rich sand is derived from weathered limestone deposits, classified as Eutric Cambisol (Soil Atlas of Europe (2005)), and is well-drained. The area was grazed during late spring, summer and early autumn by cattle (0.5 animals ha^{-1}). It was mainly free from trees and shrubs, and was dominated by herbaceous grassland vegetation.

Samples were collected along a gradient of a SE facing slope. Starting at the dry top of the gradient, the continuing slope downwards was characterized by high soil disturbance, with only 20% of the ground covered by living vegetation. At the bottom of the slope the ground was moister and less prone to drought, and the vegetation cover increased to 100%.

Soil samples were collected at 8 occasions between 2010 and 2011 (March 31, June 1, July 2, August 12, October 8 and December 3 in 2010, and February 1 and March 30 in 2011) with a soil corer (3 cm diameter) to a depth of 5 cm. Along a 48 m transect down the gradient, two subsamples were combined into one composite sample every second meter, resulting in 24 samples on each occasion. Litter, visible plant roots and pebbles were removed from the cores. Microbial growth analyses were performed within 24 h (stored at 5 °C), and all measurements were then conducted at 22 °C, in short-time laboratory assays.

Soil moisture was measured gravimetrically at all occasions as water loss after freeze drying, and organic matter (OM) as loss on ignition after 5 h at 550 °C. Ammonium (NH_4) and nitrate (NO_3) content was extracted in 0.2 M $BaCl_2$ (2:10 w:v) and phosphorus (P) availability using Bray1-P (1:10 w:v, 0.03 M NH_4F + 0.025 M HCl), then measured using flow injection analysis, FIA (ISO 11732:2005, ISO 13395) on samples that were collected the 31st of March and 8th of October 2010. Soil pH (H_2O) was measured (1:5 w:v) in samples collected the 31st of March 2010. Temperature was used as a proxy for season, as it is one important factor varying with season. The temperature was estimated as the mean daily temperature, 5 days prior to sampling day and the sampling day, at the weather station Skillinge (64290), situated 26 km from the study site (Swedish Meteorological and Hydrological Institute).

2.2. Lipid extraction, PLFA and NLFA analysis

Fatty acid analysis (according to van Aarle et al. (2003)) was used as an indication of biomass of AM fungi, saprotrophic fungi and bacteria. Soil was kept frozen and freeze-dried prior to analysis. To extract fatty acids, 5 g (dry weight) soil was mixed with a 10 mL one-phase mixture of chloroform, methanol and citrate buffer.

The fatty acids used as biomarkers for biomass were: sum of PLFA i15:0, a15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 10Me18:0 and cy19:0 for bacteria; PLFA 18:2ω6,9 for saprotrophic fungi (Frostegård and Bååth, 1996); NLFA 16:1ω5 for arbuscular mycorrhizal fungi (Olsson et al., 1995, 1998). Total microbial biomass was estimated as the sum of 31 PLFAs. Phospholipids and neutral lipid fatty acid data was presented as nmol PLFA or NLFA g^{-1} OM. The study site for this investigation had some oaks present at the top of the slope, while trees were absent in the rest of the gradient investigated. The phospholipid fatty acid 18:2ω6,9 is also