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Fungal/bacterial ratios in grasslands with contrasting nitrogen management

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Abstract

It is frequently hypothesised that high soil fungal/bacterial ratios are indicative for more sustainable agricultural systems. Increased F/B ratios have been reported in extensively managed grasslands. To determine the shifts in fungal/bacterial biomass ratio as influenced by grassland management and to find relations with nitrogen leaching potential, we sampled a two-year-old field experiment at an organic experimental farm in the eastern part of The Netherlands. The effect of crop (grass and grass-clover), N application rate (0, 40, 80, 120 kg N ha⁻¹) and manure type (no manure, farm yard manure and slurry) on the F/B ratio within three growing seasons was tested, as well as relations with soil and crop characteristics, nitrate leaching and partial N balance. Biomass of fungi and bacteria was calculated after direct counts using epifluorescence microscopy. Fungal and bacterial biomass and the F/B ratio were higher in grass than in grass-clover. The F/B ratio decreased with increasing N application rate and multiple regression analysis revealed a negative relationship with pH. Bacterial activity (measured as incorporation of [³H]thymidine and [¹⁴C]leucine into bacterial DNA and proteins) showed the exact opposite: an increase with N application rate and pH. Leaching increased with N application rate and was higher in grass-clover than in grass. Partial N balance was more positive at a higher N application rate and showed an inverse relationship with fungal biomass and F/B ratio. We conclude that the fungal/bacterial biomass ratio quickly responded to changes in management. Grasslands with higher N input showed lower F/B ratios. Grass-clover had a smaller fungal biomass and higher N leaching than grass. In general, a higher fungal biomass indicated a lower nitrogen leaching and a more negative partial N balance (or smaller N surplus), but more observations are needed to confirm the relationship between F/B ratio and sustainability.

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1. Introduction

Organic matter plays a key role in many soil processes because it affects, among others, soil structure, nutrient dynamics and soil life. Decomposition of soil organic matter is a highly important process. The rate of decomposition depends on environmental conditions (e.g. temperature, moisture conditions), on the quality (e.g. C/N ratio) of the substrate and on the characteristics of the decomposing organisms and their predators (i.e. C/N ratio, growth efficiency) (Bloem et al., 1997; Swift et al., 1979).

The main decomposition pathways in soil are either bacterial-based or fungal-based. Both bacteria and fungi support their own chain of soil fauna (De Ruiter et al., 1993; Wardle and Lavelle, 1997). Therefore, the biomass of fungi compared to bacteria can be considered as an indicator for the activity of two pathways of the soil food web, formed by fungivores or bacterivores and their predators, respectively.

Generally, fungal biomass is found to be greater than bacterial biomass in agricultural soils (Anderson and Domsch, 1975; Sakamoto and Oba, 1994; Schnurer et al.,

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1986; Zelles et al., 1995). In The Netherlands, however, analyses of soil samples from conventionally managed arable as well as grassland soils have shown that the soil microbial biomass is usually strongly dominated by bacteria (Bloem et al., 1994; Hassink et al., 1993; Velvis, 1997). Less than 20% of the soil microbial biomass in these soils consists of fungi. Consequently, the soil fauna is dominated by bacterivores and their predators (De Ruiter et al., 1993). Recent analyses of more extensively managed Dutch grasslands show a much higher contribution of fungi to the microbial biomass (50–80%) (Bloem et al., 2004).

Increased fungal/bacterial (F/B) biomass ratios in extensively managed grasslands are consistent with recent other reports (Bailey et al., 2002; Bardgett and McAlister, 1999; Donnison et al., 2000; Grayston et al., 2001; Zeller et al., 2001). The mechanisms responsible for shifts in the soil microbial community remain largely unknown. Some studies have shown that on arable land soil management affects the F/B biomass ratio (Beare et al., 1997; Frey et al., 1999). In most cases bacteria dominate under conventional tillage, whereas fungi dominate under notillage. This has been attributed to a direct contact between bacteria and substrate under conventional tillage, encouraging bacterial growth (Beare et al., 1997). Also, mycelial networks are destroyed by tillage. The effect of "tillage" (cultivation or slit injection of slurry) on fungal/bacterial biomass ratios in grasslands has not been investigated. Grazing has been reported to have either a positive (Bardgett et al., 1997) or a negative impact (Ghani et al., 2003) on the F/B ratio. Shifts in the F/B ratio related to grassland management have so far been attributed to quantity (Mawdsley and Bardgett, 1997) and quality (Grayston et al., 2001) of root exudates, changes in quality and quantity of litter or input of animal faeces (Bardgett et al., 1996) and plant productivity and composition (Donnison et al., 2000). F/B ratios may also be affected by other factors, e.g. toxic metals (Tobor-Kaplon et al., 2005). Most of these factors are related to nutrient availability. Bittman et al. (2005) found a decreasing fungal biomass as a consequence of application of manure and fertiliser. Inorganic nitrogen fertilisation has been reported to reduce the F/B biomass ratio (Bardgett et al., 1999b; Bloem et al., 2004), while organic matter with a high C/N ratio stimulates fungal growth and thus increases the F/B ratio (Alexander, 1977; Henriksen and Breland, 1999; Vinten et al., 2002). pH has been seen to have either a positive or a negative effect on F/B ratio (Bååth and Anderson, 2003; Blagodatskaya and Anderson, 1998).

Higher fungal/bacterial (F/B) biomass ratios are suggested to be indicative for a more sustainable agroecosystem with lower impact on the environment, in which organic matter decomposition and N mineralisation dominate the provision of plant nutrients for crop growth (Bardgett and McAlister, 1999; Bardgett et al., 1999a; Beare et al., 1992; Yeates et al., 1997). Because of the higher C/N ratio of fungi compared to bacteria (10 vs. 4), grazing by fungivores results in a lower N mineralisation rate than grazing by bacterivores. In addition, fungalfeeding fauna generally have a smaller biomass and lower turnover rates than bacterial-feeding fauna (Didden et al., 1994; Zwart et al., 1994). A fungi-dominated food web may therefore result in a lower N-mineralisation rate. This, however, does not necessarily lead to a lower crop production. The biomass of mycorrhizal fungi probably increases at lower soil nutrient contents (Mäder et al., 2000; Smith and Read, 1997). Their contribution to nutrient uptake may counterbalance the negative effects of a low nutrient availability to the crop and thus reduce nutrient losses to the environment (Jeffries and Barea, 1994; Smith and Read, 1997).

If an increased F/B biomass ratio has a positive effect on crop nutrient uptake efficiency and nutrient retention it is desirable to get a handle on management practices and soil properties that increase this ratio. The aim of this study was, therefore, to find out which management practices and soil characteristics affect the F/B biomass ratio in a Dutch grassland agroecosystem, and whether the F/B ratio can be used as an indicator for a system with low nutrient losses.

We hypothesise an increased fungal biomass and/or F/B ratio: (1) at lower availability of inorganic N; (2) as a consequence of application of manure with higher C/N ratio; (3) when farmyard manure is applied superficially compared to slit injection of slurry. We furthermore hypothesise that a higher F/B ratio reduces N leaching potential. We evaluate factors related to fertiliser regime and management, i.e. plant species composition and organic matter characteristics together with pH as predictors for the F/B biomass ratio or fungal and bacterial biomass alone.

2. Materials and methods

2.1. Sampling site

A field trial was done in a pasture of the organic experimental farm "Aver Heino" at Heino in the eastern part of The Netherlands ($52^{\circ}25'$ north and $6^{\circ}15'$ east), which was sown with a grass-clover mixture in 1997. After a period of grazing and mowing the field trial was established in 2001. The humid sandy soil was classified as a gleyey sand with a semi-permeable loam horizon at 70–80 cm.

The trial consisted of seven rows, corresponding to three manure treatments: no manure (one row), farm yard manure (three rows) and slurry (three rows). The three rows of farm yard manure and slurry received manure at three different N application rates: 40, 80 and 120 kg N ha^{-1} . Each of the seven rows was subdivided into nine subplots which differed in crop: grass (*Lolium perenne* L.) or grass-clover (*Lolium perenne* L. and *Trifolium repens* L.). Dimensions of the subplots were $15 \text{ m} \times 2.7 \text{ m}$, bordered by a 0.3 m bufferzone. In each row a number of

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