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Responses of Collembola and Protura to tree girdling – some support for ectomycorrhizal feeding

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Abstract

Ectomycorrhizal (EM) fungi are very common in forest soils, but their role as a food resource for fungivorous soil animals is poorly known. We used two tree-girdling experiments in *Picea abies* forests and one experiment in a *Pinus sylvestris* forest, all in northern Sweden, to indirectly test if Collembola and Protura in boreal forest soils prefer EM fungi over other fungi. We assumed that tree girdling will stop the flux of carbohydrates to roots and associated fungi, and thereby inhibit growth and long-term survival of EM fungi. After about one year, proturans decreased in abundance after girdling, indicating that they prefer feeding on EM fungi, while most collembolan species seemed to be unaffected by girdling and the presumed reduction in EM fungi. However, the collembolans *Mesaphorura macrochaeta*, *Anurida granaria* and *Parisotoma notabilis* increased in abundance after girdling in one of the three experiments, and *Micranurida pygmaea* decreased. With the exception of the latter species, this is in accordance with the common opinion that most Collembola prefer saprotrophic fungi over EM fungi, while Protura are at least partly dependent on EM fungi.

Key words: ectomycorrhiza, food-choice, microarthropods, mycorrhizal fungi, field experiment.

1. Introduction

In boreal forests, where tree growth is normally limited by nitrogen, the surface of virtually all roots are covered by a thick mantle of ectomycorrhizal (EM) fungi (Smith & Read 1997), and as much as 80 % of the fungal biomass in boreal forest soils can consist of EM fungi (Wallander et al. 2001, 2003, Högberg & Högberg 2002). Even though EM fungi are very common in forest soils, multiple choice feeding experiments often show surprisingly similar feeding preferences by fungivores over a wide range of taxa, with plant pathogens being among the most preferred fungal diet while mycorrhizal fungi are less preferred (Maraun et al. 2003, Bonkowski et al. 2009). However, recent studies of Oribatida in boreal forest soils show that some species are favoured by the presence of EM fungi (Remén et al. 2008, 2010) and some species on Oribatida have been shown to prefer EM fungi in food-choice experiments (Schneider et al. 2005). Another group that is known to feed on mycorrhizal fungi is proturans (Sturm 1959, Nosek 1977).Proturans are an often neglected group of soil animals that are especially common in the rhizosphere of trees with mycorrhiza (Nosek 1977). Collembolans, on the other hand, are normally found to prefer other fungi than mycorrhizal fungi in food-choice experiments (e.g. Hiol Hiol et al. 1994, Klironomos & Kendrick 1996, Klironomos & Ursic 1998).

Most studies regarding collembolan food choice have been made with arbuscular mycorrhizal (AM) fungi (e.g. Larsen & Jakobsen 1996, Klironomos & Kendrick 1996, Klironomos & Ursic 1998). Also, most of the experiments concerning food choice of microarthropods have been done in the laboratory. There are certain disadvantages with this method. It is impossible to present more than a tiny fraction of the enormous diversity of fungi that exist in the field. In addition, culture conditions influence the feeding preferences of Collembola (Leonard 1984, Sadaka-Laulan et al. 1998). The study of food choice of soil fauna in the field is difficult due to the opaque nature of the soil in combination with the small size of the study organisms.

Tree-girdling has been used as a method to distinguish between autotrophic (roots and associated mycorrhizal fungi) and heterotrophic (microbes) soil respiration (Högberg et al. 2001, Olsson et al. 2005). Girdling selectively removes the supply of host-derived assimilates that EM fungi are dependent on but has no immediate effect on the supply of carbon compounds to saprotrophic fungi from organic substrates. The method has also been used to separate mycorrhizal feeders from other fungivores among Oribatida (Remén et al. 2008), and we used the same three already established girdling experiments as Remén et al. (2008) to investigate if there were species favoured by EM among Collembola and Protura. Our hypothesis was that we would see a decline in Protura abundances, since at least some species are known to feed on EM fungi, while Collembola that seem to prefer other food sources would be less affected.

2. Materials and methods

Samples were taken from three already established girdling experiments in northern Sweden, Åheden, Storskogberget and Flakaliden, all sites situated relatively close to each other (lat. 64 °N; long. 19-21 °E) (Remén et al. 2008). Åheden is a pure Scots pine (*Pinus sylvestris* L.) site, where the stand is 45-55 years-old with a sparse field layer of *Vaccinium vitis-idaea* (L.), *V. myrtillus* (L.) and *Calluna vulgaris* (L.) Hull. Three treatments, early girdling (EG), late girdling (LG) and ungirdled control forest (C) were established in 2000. Tree-girdling involves stripping the stem bark to the depth of the current xylem at breast height (see Högberg et al. 2001 for further details). Storskogberget (Göttlicher et al. 2008) had a 120-year-old stand of Norway spruce (*Picea abies* (L.) Karst.) with a field layer of mainly *V. myrtillus*. Flakaliden (Olsson et al. 2005) had a 40-year-old stand of Norway spruce with a field layer of *V. vitis-idaea*, *V. myrtillus* and *Deschampsia flexuosa* (L.) Trin. The latter two stands had girdled (G) and control (C) plots.

The experiment at Åheden consisted of nine 30 m x 30 m plots arranged in three separate blocks (each block containing three plots with different treatments) with 1300 trees hectare^{-1} (Högberg et al. 2001). The experiment at Storskogberget consisted of six circular plots with a radius of 15 m and a tree density of 1080 hectare^{-1} (Göttlicher et al. 2008). Three pairs of girdled and non-girdled plots formed three blocks. The experiment at Flakaliden consisted of eight 50 m x 50 m plots with four pairs of girdled and non-girdled plots forming four blocks.

Microarthropods were sampled at Åheden in late May 2001, 11 months after early girdling and 9 months after late girdling. The samplings at Storskogberget and Flakaliden were made in October 2003, 16 months after girdling. Three soil samples, each with a surface area of 100 cm^2, were taken to a depth of 10 cm including the organic (LFH) and the upper 2 cm of the bleached mineral soil (E) layers.

The animals were extracted in modified Tullgren funnels equipped with lamps for heating up to 45 °C at the end of the extraction, and were collected in 70-80% ethanol. Collembolans were determined to species using the keys by Fjellberg (1980, 1998, 2007) and proturans were determined according to Nosek (1978) and Copeland & Imadaté (1990).

Data on individual numbers and species numbers were analysed to obtain mean and total values per plot, respectively. The data were based on three subsamples per plot. Because of the non-normal distribution, all data were transformed using ln(x+1), where x is the mean number of individuals per plot. Data were analysed using a general linear model with block and treatment as class factors. The GLM procedure in SAS for Windows, version 9.1 was used. The ANOVA was followed up by pre-planned, pair-wise comparisons between the different treatment means.

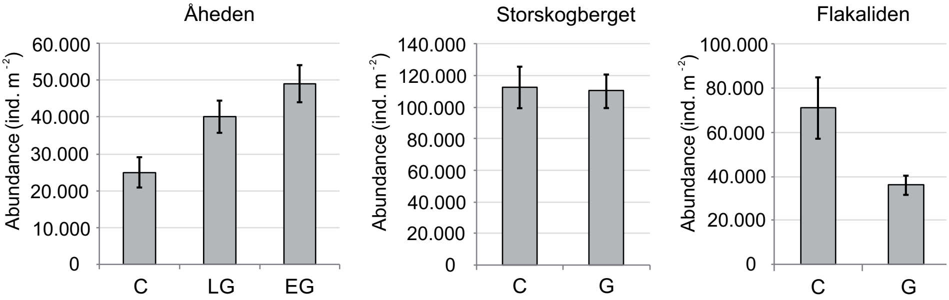

Fig. 1 Mean total abundances (±SE) of Collembola at Åheden, Storskogberget and Flakaliden in control (C) and girdled (G) plots. At Åheden, girdling in June was called early girdling (EG) and in August late girdling (LG).

3. Results

The total abundances of Collembola increased (ANOVA; p = 0.019) in girdled plots at Åheden, and the abundances were higher in plots with early than with late girdling (Fig. 1). At Storskogberget (Fig. 1) the total abundance of Collembola was very similar in girdled and control plots, while the total abundances were lower in girdled than in control plots (ANOVA; p = 0.021) at Flakaliden (Fig. 1). The three most common species were *Mesaphorura macrochaeta*, *Isotomiella minor* and *Anurophorus septentrionalis* at Åheden, *Pseudanurophorus binoculatus*, *Willemia anophthalma* and *Micraphorura absoloni* at Flakaliden, and *Mesaphorura* sp., *W. anophthalma* and *M. absoloni* at Storskogberget. Only a few species responded with significant changes to girdling (Tab. 1). Three species (*M. macrochaeta*, *Anurida granaria* and *Parisotoma notabilis*) responded with an increase in number after girdling, but only at one of the three sites. One species, *Micranurida pygmaea* actually occurred in lower abundance in girdled than in control plots at Flakaliden.

The abundance of Protura was lower in girdled plots than in control plots (p = 0.048) at Storskogberget (Fig. 2), whereas there was no significant difference between the numbers of proturans between the treatments at Flakaliden. However, the mean numbers were lower in girdled plots (Fig. 2). At Åheden, only two specimens of Protura were found, and this site was therefore not further analysed with regard to proturans. All proturans belonged to the genus *Eosentomon*.

Tab. 1 Collembolan species with significant differences in abundance (SE within parentheses) between the control (C) and girdling (G) treatments at three sites. Åheden had separate plots for early girdling (EG, June) and late girdling (LG, August). Åheden and Storskogberget had 3 blocks (replicates) and Flakaliden had 4 blocks. P-values given according to an ANOVA.

Site	Species	Treatment	Abundance (ind. m^{-2})	p-value
Åheden (*Pinus sylvestris*)	*Mesaphorura macrochaeta*	C	7200 (1200)	0.001
		LG	15100 (3200)	
		EG	16000 (3000)	
Storskogberget (*Picea abies*)	*Anurida granaria*	C	220 (30)	0.042
		G	480 (80)	
	Parisotoma notabilis	C	480 (120)	0.025
		G	3000 (770)	
Flakaliden (*Picea abies*)	*Micranurida pygmaea*	C	17300 (4400)	0.008
		G	4400 (580)	

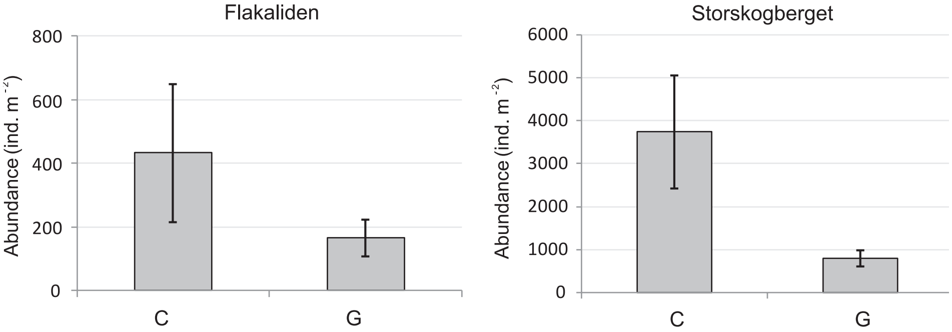

Fig. 2 Mean abundances (±SE) of Protura (*Eosentomon* sp.) at Storskogberget and Flakaliden in control (C) and girdled (G) plots.

4. Discussion

The significant decline of proturans after tree girdling at Storskogberget supports the idea that this group can feed on mycorrhizal fungi (Sturm 1959, Nosek 1977, Krauss & Funke, 1999) and can even prefer EM fungi over other fungal food choices. However, there are also less supportive data for this idea, for example from clear-cuts. In a study of soil faunal responses to clear-cutting, no significant effect was found on proturans for several years (Malmström et al. 2009). Here, the decrease in proturans was only significant at one site. This could be due to the low power of our study (n = 3), but also to the possibility that *Eosentomon* species can feed on both mycorrhizal and saprotrophic fungi (Sturm 1959). It is also possible that the larger diversity of dwarf-shrubs (with ericoid mycorrhiza) and other ground vegetation (with arbuscular mycorrhiza) at Flakaliden can support more viable populations of proturans at Flakaliden after tree-girdling (when EM fungi declines) than at Storskogberget with mostly *V. myrtillus* (Högberg et al. 2009) and its ericoid mycorrhiza.

Collembola showed a mixed response to tree-girdling, decreasing in abundance only at Flakaliden. This decline is probably not a sign of EM dependence, since many of the species also occurred at the other sites, and they did not decrease at these localities. The increase of Collembola at Åheden is probably due to the death of roots and associated mycorrhiza that are known to happen after tree-girdling (Bhupinderpal-Singh et al. 2003). Root and root exudates contribute significantly to the nutrition of soil animals (Gunn & Cherrett 1993, Albers et al. 2006, Pollierer et al. 2007). Ponge (2000) found that mycorrhizal fungi made up a significant part of the gut content of *M. macrochaeta* in a beech forest in Belgium, but this species actually increased in number in girdled plots at Åheden, indicating that this species may be able to feed on live mycorrhizal fungi although it is probably not dependent on it. Åheden was less disturbed by the girdling in general, and no notable increase in litter-fall was seen during the two first years on girdled plots, while at Storskogberget and Flakaliden considerable defoliation occurred the second year after girdling (Högberg et al. 2009, Olsson et al. 2005).

In conclusion, the data indicate that many proturans seemed to prefer living EM fungi, while most collembolan species seemed to be unaffected by the presumed reduction in EM fungi. However, the collembolans *Mesaphorura macrochaeta*, *Anurida granaria* and *Parisotoma notabilis* increased in abundance after girdling in one of the three experiments, and *Micranurida pygmaea* decreased. With the exception of the latter species, this is in accordance with the common opinion that most Collembola prefer saprotrophic fungi over EM fungi, while Protura are at least partly dependent on EM fungi.

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