Response of soil nematode communities to tree girdling in a subtropical evergreen broad-leaved forest of southwest China

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The impact of canopy photosynthates on soil microbial biomass and nematode trophic groups was studied in a subtropical evergreen broad-leaved forest by performing a large-scale tree girdling experiment. Total fungal biomass was unaffected by tree girdling. Bacterial biomass differed significantly between the girdled and control plots in the mineral soil, but was not affected by girdling treatment in the humus layer. Girdling reduced total nematode density in the humus layer. The reduced fungivorous nematode density in girdled plots in the humus layer suggested a modified energy flow through the fungal based pathways. There were no differences in the abundance of bacterial-feeding, herbivorous and omnivorous-predatory nematodes between the girdled and control plots in both humus and mineral soil layers. This study provides direct evidence that the termination of belowground photosynthetic-C allocation achieved by tree girdling affects soil nematodes, and that different trophic groups vary in their responses to the reduction of C efflux into the soil.

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1. Introduction

Terrestrial ecosystems are sustained by photosynthetic fixation of carbon above-ground. Over the past decades, studies on the importance of carbon input from above- and below-ground to soil communities have received great attention (Hättenschwiler et al., 2005). Recent studies showed that plant carbon can flow through soil foodwebs at a rapid rate (Albers et al., 2006; Ostle et al., 2007; Pollierer et al., 2007). Plants transfer photosynthetic-C to rhizosphere soil via living roots as exudates, mucilages and sloughed off cells, jointly called rhizodeposition (Johansson, 1992). This rhizodeposited C has been suggested as another important driver of soil decomposer communities in addition to the much slower fluxes of carbon arising from the decomposition of shoots and root-derived litter (Högberg and Read, 2006). However, neither the magnitude nor the mechanisms of the supply of canopy photosynthetic-C to soil biota has been fully appreciated, particularly in forest ecosystems, because of the size of the plants and the great spatial heterogeneity of the soils (Mikola and Kytöviita, 2002; Högberg and Read, 2006; Göttlicher et al., 2006).

Tracking the incorporation of current assimilated C into soil organisms through plant roots is difficult under natural soil conditions. In grassland ecosystems, defoliation and herbivores grazing were found to affect the amount of rhizodeposition and subsequently altered soil biota dynamics (Mikola and Kytöviita, 2002; Christensen et al., 2007). However, the effects of defoliation and herbivory on plant C allocation may differ among plant species by either increasing or decreasing the allocation of current assimilates to roots and root exudation (Holland et al., 1996; Wilsey et al., 1997). Carbon-based isotopic labeling techniques have been applied effectively to characterize the pathway of photosynthetically fixed C (Johnson et al., 2002; Leake et al., 2006; Ostle et al., 2007), but these techniques are generally applied in artificial growing environments or grassland/agro-ecosystems (Pollierer et al., 2007). Root exclusion by trenching is a commonly used method in forest ecosystems to suppress the energy link between trees and the soil systems and has been used to assess the photosynthesis controls on soil microbial and faunal communities (Simard et al., 1997; Siira-Pietikäinen et al., 2001; Brant et al., 2006). However, this method destructively disturbs soils and terminates the process of plant uptake of water and nutrients.

In recent years, a number of studies have investigated the effects of eliminating photosynthetic translocation to belowground on soil processes by physical girdling of trees. Tree girdling instantaneously terminates the flux of photosynthates from tree canopy through the phloem to tree roots, while there is minimal immediate disturbance to the soil and roots (Högberg et al., 2001). Studies from Aheden and Flakaliden in Sweden (e.g. Högberg et al.,
researchers have suggested that the roots in the girdled plots
exudation to the soil (Högberg et al., 2001), though several
the fact that girdling can prevent the translocation of photosyn-
their abundance will also decrease if the abundance of lower
girdling due to their high hierarchical position in the food web, but
labile substrates and the microbial activities are suppressed after
when the plants respond to girdling by decreasing exudation of
consumers, bacterivores and fungivores will decrease in abundance
depend on current assimilates belowground; (ii) as secondary
nematodes are expected to decrease because they also strongly
girdling; (iii) as tertiary (and higher) consumers, omnivorous and
Among soil biota, nematodes possess attributes that are useful
to reflect the responses of multi-trophic level soil organisms on tree
girdling. Nematodes are one of the most abundant groups of soil
habitants and react quickly to environmental changes (Bongers
and Ferris, 1999). Furthermore, nematodes appear in a wide array of
trophic groups (herbivores, bacterivores, fungivores, omnivore-
predators) reflecting the current or recent availability of their C
sources, and their abundance is assumed to mirror that of other
important consumers in soil (De Deyn et al., 2004). Hence nema-
todes may be expected to respond both directly to changes in plant
carbon allocation to the belowground environment, and indirectly
to changes in microbial communities that respond directly to plant
carbon inputs (Neher et al., 2004). In this study, we examined the impact of photosynthates on soil
nematodes at different trophic levels of an evergreen broad-leaved
forest of southwest China. We performed a large-scale tree girdling
experiment and analyzed the changes of soil microbial biomass and
nematode community structure. We hypothesized that the
decreased C allocation to belowground induced by tree-girdling
will affect soil microbial biomass and nematode trophic groups: (i)
as primary consumers, the bacterial biomass will decrease after
girdling due to the decreased root exudates in soils, the biomass of
fungi will decrease after girdling because of the direct elimination
of the root-mycorrhizal network, and the abundance of herbivorous
nematodes are expected to decrease because they also strongly
depend on current assimilates belowground; (ii) as secondary
consumers, bacterivores and fungivores will decrease in abundance
when the plants respond to girdling by decreasing exudation of
labile substrates and the microbial activities are suppressed after
girdling; (iii) as tertiary (and higher) consumers, omnivorous and
predacious nematodes are expected to be the least responsive to
girdling due to their high hierarchical position in the food web, but
their abundance will also decrease if the abundance of lower
trophic level groups decreases. The above hypotheses are based on
the fact that girdling can prevent the translocation of photosyn-
hathe-C from tree canopy to the roots and decrease the input of root
exudation to the soil (Högberg et al., 2001), though several researchers have suggested that the roots in the girdled plots
eventually die being an input of resources for decomposers (Hög-
berg et al., 2001; Högberg and Högberg, 2002; Binkley et al., 2006).

2. Materials and methods

2.1 Site description and experimental design

The study was conducted in a subtropical evergreen broad-
leaved forest in Xujiaba (24°32′N, 101°01′E; altitude 2476 m above
sea level) which is located at the Ailao Mountains in southwestern
China. The soil is acidic yellow-brown loam (pH 4.2–4.5) with a
humus layer of 7–12 cm in thickness (Chan et al., 2006). The
organic carbon content, nutrient content and exchangeable cations
are higher in the humus layer than in the mineral soil (Chan et al.,
2001; Bhopinderpal-Singh et al., 2003; Giesler et al., 2007),
Wetzstein of Germany (Subke et al., 2004; Hahn et al., 2006; Ekberg
et al., 2007), Bahia of Brazil (Binkley et al., 2006), Pura of Switzer-
land (Frey et al., 2006), North Carolina of USA (Johnsen et al., 2007)
have clearly shown that as much as half of the soil respiration was
reduced after large-scale tree girdling, which is interpreted as a
disruptive effect of girdling on root respiration and ectomycor-
rhizal activities. However, whether and how tree girdling affects
the composition of soil organisms at multiple trophic levels of soil
biota remains mostly unexplored. Schulze et al. (2005) reported
a post-girdling reduction in the numbers of proteins by 50% based on
indirect proteomic fingerprint evidence, which implied that the
current photosynthates allocated belowground may influence
different soil taxonomic groups (Högberg and Read, 2006).

Eight quadrat plots (20 × 20 m) were established in early
February 2004. To prevent ingrowth of external roots, each plot was
trenched along the four sides to a depth of 40 cm and plastic sheets
were buried into the soil. For randomly selected four plots, all the
trees (>2 cm in diameter) were girdled by removing a 5–10 cm
length of bark and phloem around the circumferences of the stems
at a height of 1.5 m above the ground on the 10th and 11th of
February. Other four plots were left ungirdled as controls.

2.2 Soil sampling and analyses

Within each of the eight plots, one subplot of 2 × 3 m in size was
designed for soil sampling. Soil samples were collected on 15
February, 15 April, 20 June, 18 August and 7 December of 2004, and
3 March of 2005, corresponding to 4, 63, 129, 188, 299 and 388 days
after girdling treatment. In each subplot, one humus sample and
one mineral soil sample were collected. We collected humus
samples using a wooden frame (20 × 20 cm). Mineral soils were
collected to a depth of 10 cm using a core sampler of 5 cm in
diameter. Each humus or mineral soil sample was then divided into
three subsamples. One subsample (ca. 5 g) was used for soil
microbial analysis, the second one (ca. 20 g) for measuring soil
water content, and the third (ca. 50–100 g) for nematode
community analysis. Total fungal biomass was estimated by meas-
uring the length and diameter of hyphae using the agar film
technique (Lodge and Ingham, 1991). The Fungal biovolume was
converted to biomass C by assuming a hyphal density of 0.33 g (dry
weight)/cm³ and 47% C content (Van Veen and Paul, 1979). Total
biomass was determined by counting the numbers and measuring
the diameters of bacteria stained with fluorescein isothiocyanate
(FITC) (Babiuk and Paul, 1970). The conversion of bacterial
cell counts to biomass C was made by a cell density of 0.3 g
(dry weight)/cm³, and 45% C content (Van Veen and Paul, 1979).
Nematodes were extracted by flotation in Ludox™ (Griffiths et al.,
1990). Nematodes were counted under a dissecting microscope
and their densities were expressed as number of individuals per 10 g
dry soil. After counting the total number of individuals, nematode
specimens were slowly dehydrated in glycerol and prepared on
slides. About 100 specimens per sample were randomly selected
and identified to genus, and classified into four functional groups
representing three trophic levels in the soil food web (Yeates et al.,
1993): primary consumers (herbivores), secondary consumers
(fungivores and bacterivores) and tertiary consumers (omnivore-
predators). Daily averages of soil temperature and volumetric
water content at 10-cm soil depth were automatically monitored with
data loggers during the experimental period.

For soil temperature, soil water content, microbial and nema-
tode variables (including biomass and abundance data), repeated
measure analysis of variance was used to test for the effects of
girdling treatment and sampling time. When the effects were
significant, multiple comparisons were made based on least square
means. Significance levels were set at alpha < 0.05. To meet
assumptions of normality and homogeneity of variance, the
biomass and abundance data were arcsine, square root or log10 \((x + 1)\) transformed if necessary.

3. Results

Soil temperature was highest in August and June 2004 and lowest in March 2005 (Fig. 1a). During the experimental period, soil temperature was not significantly affected by girdling (repeated measure ANOVA: \(F = 0.195, P = 0.677\)). Soil water content was highest during the period from April to August 2004 and lowest in winter and early spring (Fig. 1b). Although the soil water content tended to be lower in the plots with girdled trees, it did not differ significantly between the girdled and control plots (repeated measure ANOVA: \(F = 1.304, P = 0.305\)).

Mean fungal biomass ranged between 312.5 and 822.3 \(\mu\)g C/g dry soil in the humus layer (Fig. 2a) and between 163.9 and 420.6 \(\mu\)g C/g dry soil in the mineral soil (Fig. 2b) across all samples. Fungal biomass was not significantly affected by girdling in both humus and mineral soil layers (Table 1). Mean bacterial biomass in the humus layer exhibited a similar temporal fluctuation pattern as that in the mineral soil (Fig. 2c,d), and ranged from 179.0 to 555.1 \(\mu\)g C/g dry soil in the humus layer over the entire experiments and from 157.3 to 339.6 \(\mu\)g C/g dry soil in the mineral soil. In the mineral soil, bacterial biomass differed significantly between the girdled and control plots, but was not significantly affected by girdling treatment in the humus layer (Table 1).

A total of 58 nematode genera were found for all the collected samples (Supplementary Table 1). Total nematode density averaged 751–2285 individuals/10 g dry soil in the humus layer and 266–1500 individuals/10 g dry soil in the mineral soil across all samples (Fig. 3a,b), showing significant time effects in both layers. In the humus layer, girdling caused a significant decrease in total nematode density (Table 1), up to 46% reduction in girdled plots compared to control plots at the end of the experiment (Fig. 3a). In the mineral soil, the reduction of total nematode density resulted from girdling treatment was not significant (repeated measure ANOVA: \(F = 2.867, P = 0.141\)) (Fig. 3b).

Responses of soil nematodes to girdling treatment varied among different trophic groups (Fig. 3). Herbivore density was unaffected by girdling treatment in both mineral and humus layers. The density of fungivores in the humus layer was significantly affected by girdling (repeated measure ANOVA: \(F = 12.706, P = 0.012\)). In the mineral soil, the fungivore density of the girdled plots was lower than that of the control plots, but the difference was modest (repeated measure ANOVA: \(F = 5.188, P = 0.063\)). Density of bacterivores and omnivore-predators were unaffected by girdling in both soil layers.

4. Discussion

4.1. Influences of tree girdling on fungi and fungivorous nematodes

Girdling terminates the direct flow of photosynthetic-C to roots and soil systems and should have negative effects on mycorrhizal fungi which receive carbon in the form of photosynthates directly from host plants (Högberg et al., 2001; Högberg and Read, 2006). The girdling-induced reductions in mycorrhizal fungi biomass have been confirmed by many previous studies (Högberg et al., 2001, 2007; Högberg and Högberg, 2002; Högberg, 2006). The reduced mycorrhizal biomass is supposed to result in a subsequent decrease in the total fungal biomass, though the numbers of saprotrophic fungi may remain unchanged in the girdled relative to the control plots (Subke et al., 2004; Högberg et al., 2001, 2007). Such a decline of total fungal biomass in girdled plots was not detected in our study by means of direct measurements; however, decreased numbers of fungivorous nematodes possibly reflected a decreased fungal activity or fungal reproduction especially in the humus layer. Since the biomass of microbivores has been suggested to reflect the variations in rhizodeposition more sensitively than the microorganisms themselves (Christensen et al., 2007), the reduced abundances of fungivorous nematodes in the girdled plots suggest a decreased allocation of C flowing through the fungal-mediated energy pathway.

4.2. Influences of tree girdling on bacteria and bacterivorous nematodes

In the humus layer, the bacterial biomass was found unaffected by tree girdling in our study. This result is consistent with other girdling experiments where bacterial abundance was not responsive to tree girdling (Högberg et al., 2007). Other carbon input manipulation experiments such as root exclusion and aboveground insect herbivory also yielded similar results that bacterial biomass weakly correlates with plant rhizodeposition (Siira-Pietikäinen et al., 2001, 2003; Brant et al., 2006; Christensen et al., 2007). Although changes in bacterial populations were generally not detected, some studies have revealed a tight coupling between the carbon transport belowground and the population size of bacterivorous microfauna such as protozoa or nematodes (Neher et al., 2004; Allen et al., 2005). In this study, however, the abundance of bacterivorous nematodes was unaffected by girdling treatment, though the bacterial biomass in mineral soil of the girdled plots significantly decreased. This unchanged bacterivorous nematode
populations after girdling might suggest a relatively unchanged energy flow through the bacterial-mediated pathway.

4.3. Influences of tree girdling on herbivorous nematodes

Belowground herbivores feed on roots and rely on root carbohydrates. Girdling blocked the phloem flux of photosynthate-C from the tree canopies to the roots and is supposed to reduce the populations of herbivorous nematodes. Contrary to our hypothesis, the abundance of herbivorous nematodes which were dominated by ectoparasitic species such as *Criconema* and *Ogma*, did not significantly differ between girdled and control plots.

4.4. Implications from the tree girdling influences

Application of proteomic analysis revealed a dramatic post-girdling reduction in the numbers of proteins at Wetzstein, indicating a decreased abundance of most soil organisms in girdled plots (Schulze et al., 2005). Using isotopic labeling methods, Albers et al. (2006), Ostle et al. (2007) and Pollierer et al. (2007) demonstrated that the root-derived C was incorporated into virtually all decomposer animals such as mites, collembolans, earthworms, enchytraeids and isopods in the arable, grassland and forest soils. Consistent with these findings, our study shows that the termination of the belowground C supply by tree girdling leads to a prominent decrease in the abundance of total soil nematodes which is statistically significant in the humus layer. This result supports the recent views that forest soil animals can acquire carbon from belowground via roots (Pollierer et al., 2007).

Tree girdling is generally conducted to separate soil autotrophic (mycorrhizal root) from heterotrophic respirations without physical disturbing soils, because it is considered to stop the autotrophic respiration while the heterotrophic respiration remains unchanged for a short period of time (Högberg et al., 2001; Binkley et al., 2006).

### Table 1

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Error df</th>
<th>Treatment (1 df)</th>
<th>Time (5 df)</th>
<th>Treatment × Time (5 df)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Humus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal biomass</td>
<td>30</td>
<td>0.017 (0.901)</td>
<td>3.299 (0.017)</td>
<td>0.741 (0.599)</td>
</tr>
<tr>
<td>Bacterial biomass</td>
<td>30</td>
<td>0.750 (0.420)</td>
<td>26.995 (&lt;0.001)</td>
<td>1.309 (0.287)</td>
</tr>
<tr>
<td>Total nematode density</td>
<td>30</td>
<td>7.352 (0.035)</td>
<td>4.798 (0.002)</td>
<td>1.418 (0.246)</td>
</tr>
<tr>
<td>Herbivorous nematodes density</td>
<td>30</td>
<td>3.166 (0.126)</td>
<td>5.809 (&lt;0.001)</td>
<td>1.260 (0.307)</td>
</tr>
<tr>
<td>Fungivorous nematodes density</td>
<td>30</td>
<td>12.706 (0.012)</td>
<td>5.083 (0.002)</td>
<td>3.630 (0.011)</td>
</tr>
<tr>
<td>Bacterivorous nematodes density</td>
<td>30</td>
<td>2.258 (0.184)</td>
<td>6.687 (&lt;0.001)</td>
<td>1.907 (0.123)</td>
</tr>
<tr>
<td>Omnivorous-predatory nematodes density</td>
<td>30</td>
<td>1.034 (0.348)</td>
<td>1.329 (0.279)</td>
<td>0.874 (0.510)</td>
</tr>
<tr>
<td><strong>Mineral soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal biomass</td>
<td>30</td>
<td>0.088 (0.777)</td>
<td>3.135 (0.022)</td>
<td>2.227 (0.078)</td>
</tr>
<tr>
<td>Bacterial biomass</td>
<td>30</td>
<td>6.952 (0.039)</td>
<td>12.388 (&lt;0.001)</td>
<td>0.816 (0.548)</td>
</tr>
<tr>
<td>Total nematode density</td>
<td>30</td>
<td>2.867 (0.141)</td>
<td>8.306 (&lt;0.001)</td>
<td>0.900 (0.494)</td>
</tr>
<tr>
<td>Herbivorous nematodes density</td>
<td>30</td>
<td>0.227 (0.651)</td>
<td>2.590 (0.046)</td>
<td>1.597 (0.191)</td>
</tr>
<tr>
<td>Fungivorous nematodes density</td>
<td>30</td>
<td>5.188 (0.063)</td>
<td>2.634 (0.043)</td>
<td>1.637 (0.181)</td>
</tr>
<tr>
<td>Bacterivorous nematodes density</td>
<td>30</td>
<td>1.559 (0.258)</td>
<td>3.673 (0.010)</td>
<td>0.660 (0.656)</td>
</tr>
<tr>
<td>Omnivorous-predatory nematodes density</td>
<td>30</td>
<td>1.242 (0.308)</td>
<td>15.738 (&lt;0.001)</td>
<td>0.970 (0.453)</td>
</tr>
</tbody>
</table>

Shown are *F*-values with significance levels in parentheses. Significant effects (*P* < 0.05) are indicated in bold.
This study provides direct evidence that tree girdling also affected soil faunal groups, and that soil organisms of different trophic groups varied in their responses to tree girdling. Thus, we agree with Bhupinderpal-Singh et al. (2003) and Subke et al. (2004) that the reported values for “soil autotrophic respiration” inferred from forest girdling experiments should be understood as “root mediated” respiration rather than the contributions of mycorrhizal root only.

The main effects of girdling on soil nematode communities were generally observed in the humus layer rather than the mineral soil layer. This is probably because of the greater biomass of fungal hyphae in the upper soil layers. In a trenching experiment,
Siira-Pietikäinen et al., 2003 also found that the significant responses of microbes only occurred in the humus layer not in the mineral soil layer. This may imply that the photosynthetically active C was mainly distributed to the humus layers.

The treatment induced change in soil nematode communities was detectable during the study period; however, it was generally weaker than expected. It probably reveals only partial effects of girdling treatments in the studied forest because of the large storage of carbohydrates in roots and stems (Qiao, personal communication). Greater differences in certain nematode ecological parameters between treatments were observed at the end of the experiment than those at the early stages of the study. Therefore, longer-term studies would probably show more pronounced girdling effects on soil communities.

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Appendix. Supplementary information

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.soilbio.2008.07.031.

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