Arbuscular Mycorrhizal Fungi May Mitigate the Influence of a Joint Rise of Temperature and Atmospheric CO₂ on Soil Respiration in Grasslands

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Received 24 June 2009; Accepted 16 October 2009

Recommended by Johannes van Veen

We investigated the effects of mycorrhizal colonization and future climate on roots and soil respiration (Rsoil) in model grassland ecosystems. We exposed artificial grassland communities on pasteurized soil (no living arbuscular mycorrhizal fungi (AMF) present) and on pasteurized soil subsequently inoculated with AMF to ambient conditions and to a combination of elevated CO₂ and temperature (future climate scenario). After one growing season, the inoculated soil revealed a positive climate effect on AMF root colonization and this elicited a significant AMF x climate scenario interaction on root biomass. Whereas the future climate scenario tended to increase root biomass in the noninoculated soil, the inoculated soil revealed a 30% reduction of root biomass under warming at elevated CO₂ (albeit not significant). This resulted in a diminished response of Rsoil to simulated climatic change, suggesting that AMF may contribute to an attenuated stimulation of Rsoil in a warmer, high CO₂ world.

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1. Introduction

In grasslands the majority of the ecosystem carbon pool is stored below ground, making soil respiration (Rsoil) an important component of the carbon balance of grassland ecosystems [1]. Up to now, Rsoil remains one of the most uncertain components of carbon cycle models, ranging from process-based ecosystem models [2] to earth system models [3]. Responses of Rsoil to climate change have been well studied for single factor effects of, for example, warming, CO₂ enrichment, or changes in precipitation [4–6]. However, multifactor manipulative experiments remain scarce and the complexity of interactive effects contributes largely to the limited understanding.

Soil respiration represents the integrated CO₂ flux of root respiration, mycorrhizal respiration (often considered part of the autotrophic respiration), and heterotrophic respiration. Two key factors that control Rsoil are carbon supply and temperature [4]. Carbon supply depends primarily on plant productivity, which generally responds positively to CO₂ enrichment [5–7] mainly because of increased photosynthetic rates [8]. Via consequent increases in litterfall [9], root exudation [10], root biomass [11], and root turnover [12], carbon availability for the microbial community increases, stimulating microbial biomass and activity [6]. Moreover, root respiration probably also increases because of the increased root biomass and possibly also because of increased root respiration per unit biomass (specific root respiration) [13]. Overall, CO₂ responses of Rsoil beneath herbaceous species range between a 10% decline and a 162% increase [14]. Given that all biochemical processes are temperature dependent, warming may increase Rsoil [15] via enhanced autotrophic [16, 17] and heterotrophic respiration [17]. However, this response is often restricted to the early stage of heating experiments, after which Rsoil frequently returns to its original level [18]. Such downregulation is likely due to the depletion of labile organic carbon pools in the soil [19–22], reducing microbial activity, but thermal adaptation of
root [23, 24] and microbial activity [19] could also play an important role.

Studies on the effects of a combination of elevated CO₂ and temperature on soil dynamics are scarce and the results of the few studies examining the combined effects of warming and CO₂ enrichment are equivocal [20–22, 25, 26]. Possibly, indirect effects such as changes in soil moisture or nutrient availability are responsible for the variability among the observed responses. Moreover, also the temperature sensitivity (often expressed as Q₁₀, the factor by which Rₘ₀ may change in response to climatic change. As shown by Wan et al. [26], Q₁₀ of Rₘ₀ might decrease in response to a joint rise of temperature and atmospheric CO₂ concentration, but opposite trends were observed by Tingey et al. [25]. More research is obviously required to solve this issue.

Many manipulation experiments focusing on climate change impacts on ecosystem functioning were conducted under controlled conditions, without considering the mycorrhizal fungi living in symbiosis with the plants. However, in grassland ecosystems, most plants are associated with arbuscular mycorrhizal fungi (AMF) [27]. Hence, an important fraction of Rₘ₀ may be assigned to AMF. Despite the low decomposability of chitin—the structural backbone of fungal tissue [28]—colonization by AMF can enhance belowground respiration rates [29], although this was not observed in all studies [30]. An AMF-induced increase of Rₘ₀ could result from respiration of the biotrophs themselves, but indirect AMF effects on root exudation [31], root longevity and decomposition [32], soil aggregate stability [33], and nutrient acquisition [29] are probably crucial as well. In this context, recent research that highlighted the central role of mycorrhizal fungi may play regarding feedbacks on global change [34, 35].

Arbuscular mycorrhizal fungi also are sensitive to climate change. Elevated CO₂ concentrations can indirectly affect AMF through increased C allocation from the host plant to the fungus [36], although this effect might be overestimated under abrupt compared to gradual increases of atmospheric CO₂ [37]. A meta-analysis by Treseder [38] revealed an overall increase in mycorrhizal abundance in response to elevated CO₂. In turn, mycorrhizal fungi can affect plant and soil dynamics. In a review on CO₂ effects on plant symbiosis with mycorrhizal fungi, Diaz [39] reported that most infected plants exhibited an additional increase in dry weight and/or a better nutritional status when exposed to elevated CO₂ concentrations. Furthermore, mycorrhizal hyphae can redistribute the recently fixed carbon away from the roots. Hence, in the presence of mycorrhizal fungi, effects of CO₂ enrichment are not necessarily restricted to the rhizosphere. Moreover, the glycoprotein glomalin that is produced by AMF stimulates soil aggregation [40]. In a high CO₂ world, AMF could thus enhance soil C sequestration directly via C allocation to deeper soil and indirectly via enhanced soil aggregate stability through enhanced glomalin production [41].

Studies combining AMF and climate warming are less abundant. In one study, a temperature-induced increase in fungal growth was associated with increased specific root length [42]. Hawkes et al. [43] found that plant photosynthates were more rapidly transferred to and respired by AMF when exposed to elevated temperatures. With regard to Rₘ₀, however, interactions between warming and/or CO₂ enrichment and the AMF status of plants remain to be tested.

In a model grassland experiment, we exposed plant communities on pasteurized soil (no living AMF present) and on pasteurized soil subsequently inoculated with AMF to ambient conditions and to a joint rise of atmospheric CO₂ and temperature (future climate scenario). We investigated how Rₘ₀ and root characteristics such as root length, biomass, diameter, and C : N ratio responded to this future climate and to AMF colonization. A major objective was to test for interactions between presence of AMF and the future climate scenario.

2. Materials and Methods

2.1. Study Site and Experimental Set-Up. This study was conducted at the Drie Eiken Campus of the University of Antwerp (Wilrijk, Belgium, 51° 09′ N, 04° 24′ E). The climate of Northern Belgium is characterized by mild winters and cool summers, with average annual air temperatures (T_air) varying around 9.6 °C. Annual precipitation averages 776 mm and is more or less equally distributed throughout the year.

In May 2007, an experimental platform with artificially assembled grassland model ecosystems was established. The platform consisted of 10 sunlit chambers, each with an interior surface area of 2.25 m². The top was covered with a colorless polycarbonate plate. The sides were covered with transparent polyethylene film. Five of these chambers were exposed to ambient T_air and ambient CO₂ concentrations. The other five chambers were continuously exposed to a future climate scenario with approximately 620 ppm CO₂ and air temperatures were warmed 3 °C above fluctuating ambient T_air. All future-climate chambers had their individual CO₂ control group. The CO₂ concentration was measured every 8 seconds with an infrared gas analyzer (WMA-4, PP-Systems, Hitchin, UK) and the concentration was adjusted independently in each chamber.

This experiment was performed on 24 grassland communities, spread over the 10 chambers. Hence, each climate scenario x AMF inoculation combination was represented by six replicates. Each community contained 18 plants, with three individuals of six species, selected from three functional groups: two grass species (Poa pratensis L., Lolium perenne L.), two N-fixing dicots (Medicago lupulina L., Lotus corniculatus L.), and two non-N-fixing dicots (Rumex acetosa L., Plantago lanceolata L.). The 18 individuals were planted in a hexagonal grid with a 4.5 cm interspace between the plants. Interspecific interactions were maximized by avoiding clumping.

Each plant community was constructed in a PVC tube with a height of 40 cm and an inner diameter of 24 cm. The PVC tubes were filled to a height of 36 cm with sandy soil (89.2% sand, 8.7% silt, 2.1% clay), collected from an extensively managed grassland in Berlaar (Antwerp, Belgium). The soil was pasteurized at 90 °C, during two
successive 8-hour cycles. Subsequently, the soil was well mixed to obtain similar initial microbial communities in all communities. At the moment we transplanted the five-week-old seedlings from seedling trays to the PVC tubes, half of the communities were inoculated with 100 g inoculum, consisting of calcined clay with two AMF taxa, *Gigaspora margarita intraradices* MUCL 41833 (+/- 2 spores/g of inoculum) and *Glomus intraradices* BEG 34 (40 root fragments per g of inoculum, 85% frequency of root colonization [44]). The inoculum (5.5 g per seedling) was placed in direct contact with the root systems at transplantation. Our experiment thus consisted of four treatments: inoculated pasteurized soil at ambient temperature and CO₂ concentrations (IA), inoculated pasteurized soil at elevated temperature and CO₂ concentrations (IE), non-inoculated pasteurized soil at ambient temperature and CO₂ concentrations (NA), and non-inoculated pasteurized soil at elevated temperature and CO₂ concentrations (NE).

In order to avoid unrealistic soil temperatures, the PVC tubes containing the grassland communities were buried in the soil. During the experiment, the communities in both climate scenarios received equal irrigation amounts. Communities were watered three times per week with a drip irrigation system at a rate of 0.05 L hour⁻¹ m⁻². The amount of water supplied was based on the 10-year average monthly precipitation recorded in the nearby meteorological station of Deurne (Antwerp, Belgium). Water could freely drain from the containers, while capillary rise of groundwater was prevented by means of drainage pipes installed beneath the chambers.

### 2.2. Soil Respiration

In October 2007, five months after the start of the experiment, we measured $R_{\text{soil}}$ on all 24 plant communities of each treatment. Measurements were made on five days between 15 and 22 October. During each of these five days, we measured $R_{\text{soil}}$ in each community three times within 10 minutes, using an infrared gas analyzer (EGM-4; PP-Systems, Hitchin, UK) coupled to a small PVC chamber (8 × 5 × 1.5 cm). These PVC chambers were permanently installed on a strip of bare soil inside the communities. An aerating hole in the chambers ensured mixing with the root systems at transplantation. Our experiment thus consisted of four treatments: inoculated pasteurized soil at ambient temperature and CO₂ concentrations (IA), inoculated pasteurized soil at elevated temperature and CO₂ concentrations (IE), non-inoculated pasteurized soil at ambient temperature and CO₂ concentrations (NA), and non-inoculated pasteurized soil at elevated temperature and CO₂ concentrations (NE).

In order to avoid confounding effects through changes in plant photosynthesis [45], $R_{\text{soil}}$ was always measured in the morning (between 8 hours and 12 hours, with measurements of the different treatments randomized).

One of our objectives was to compare the temperature sensitivities of $R_{\text{soil}}$ in the different treatments. To this end, we altered the temperatures in the chambers ca. 24 hours before measuring $R_{\text{soil}}$, such that we obtained a sufficiently large soil temperature range as well as sufficient overlap in the temperature ranges between both climate scenarios. In the ambient chambers, $R_{\text{soil}}$ was measured at soil temperatures (at 5 cm depth) of, on average, 7.7, 9.2, 10.9, 12.2, and 14°C, while average soil temperatures under the future climate scenario were 7.7, 9.1, 11.1, 13.5, and 14.8°C during $R_{\text{soil}}$ measurements.

### 2.3. Soil and Root Sampling

Six months after the start of the experiment, belowground biomass was harvested. Twelve soil cores (2.0 cm diameter) were taken in four of the six plant communities of each treatment. The entire soil profile of each community was sampled and divided into four depths: 0–9, 10–18, 19–27, and 28–36 cm. In order to obtain comparable soil samples, soil cores were collected following the same pattern for each community; we sampled six cores near each of the six species present and six other cores were sampled in between three species, considering different species combinations. All 12 samples from the same depth in the same community were pooled and a subsample was used for root analyses. We washed the extracted root material by hand and stored the roots in a Ringer’s solution in the fridge (4°C). Within two weeks after sampling, roots were analyzed for root length and diameter, using WinRHIZO image analysis software (Regent Instruments Inc., Quebec, Canada). Subsequently, roots were oven dried at 70°C for 48 hours before being weighed. We determined the C and N concentrations on 5 to 7 mg roots from the top 9 cm layer, using a C/N analyzer (NC-2100, CarloErba, Italy). Further, we also analyzed 25 to 35 mg soil of each soil layer (0–9 cm, 10–18 cm, 19–27 cm, and 28–36 cm; dried at 60°C for 48 hours) of each community for C and N concentration.

### 2.4. Mycorrhizal Root Colonization

In order to quantify the degree of AMF root colonization, dried root samples (from the top 9 cm soil of four plant communities per treatment) were cleared in a 10% KOH solution (90°C for 30 minutes) and then stained with a blue ink solution (90°C for 30 minutes; 1% HCl with 2% blue ink, Parker; adapted from Vierheilig et al. [46]). Thirty randomly selected root pieces (10 mm length) of each sample were examined under a bright-field microscope at 50x or 125x magnification. The frequency and intensity of AMF root colonization were estimated according to the method described by Declerck et al. [47]. We calculated the frequency of AMF colonization as the percentage of root segments that contained hyphae, arbuscules, or vesicles. The intensity of colonization, that is, the abundance of vesicles, hyphae, and arbuscules in each colonized root segment, was estimated using different intensity classes (1%–20%, 21%–40%, 41%–60%, 61%–80%, 81%–100%) [47].

### 2.5. Potential Heterotrophic Respiration

Air-dried soil (50 g from the top 9 cm) of 18 plant communities (five samples for IA and NA, four samples for IE and NE) was rewetted to obtain a water content of 60% of field capacity and was subsequently incubated in plastic bottles (1.2 L) at 20°C. We measured respiration rates (potential heterotrophic respiration; $R_{\text{pot}}$) after 6, 11, 13, and 19 days of incubation, using an infrared gas analyzer (EGM-4; PP-Systems, Hitchin, UK), coupled to the bottles. Each sample was measured twice within five minutes and the average of both measurements was used for further analysis.

### 2.6. Data Analysis

In order to detect differences in $R_{\text{soil}}$ between our treatments, we needed to determine soil respiration at a reference temperature (i.e., the basal soil respiration;
BR). As shown by, for example, Wan et al. [26], temperature sensitivity may also change in response to climatic change. We computed the basal respiration rate and the temperature sensitivity may also change in response to climatic change. In our case, only the BR at growth conditions may give an impression of potential downregulation (i.e., lower R_{soil} under the future climate scenario than expected from measurements at ambient conditions), with homeostasis occurring if this BR is equal for both climate scenarios. Homeostasis would thus imply that climate scenario has no effect on R_{soil}. Therefore, we will only discuss differences in BR at growth conditions, that is, BR at 10°C for the ambient treatment versus BR at 13°C for the future climate scenario. The same accounts for the potential heterotrophic respiration. Therefore, we computed R_{hpot} at 23°C for the future climate scenario (for all treatments, R_{hpot} was measured at 20°C), to obtain R_{hpot} with a 3°C difference between both climate scenarios. To this end, we used a Q_{10} of two and computed R_{hpot} at 23°C for the simulated future climate scenario using (1) with b = 23°C.

Regression analyses were performed in Matlab (7.2.0.232, The Mathworks, Natick, MA, USA); statistical analyses were made in SAS (SAS system 9.1, SAS Institute, Cary, NC, USA). Except when stated differently, we used a nested two-way ANOVA to test for effects of AMF inoculation (AMF effects),

$$R_{soil} = BR \times Q_{10}^{(T-b)/10}$$

(1)

with T being soil temperature at 5 cm depth and b = 10°C for the ambient treatment or b = 13°C for the future climate scenario. Like this, we obtained BR at growth temperature and the weighted mean Q_{10}, using the inverse of the standard error (SE) on the estimated parameters as weight factors (i.e., 1/SE of BR) and 1/SE of Q_{10}) to compute the weighted mean BR and Q_{10} resp.).

In this study, with a combination of elevated temperature and CO_{2}, we cannot distinguish between thermal acclimation and acclimation to CO_{2} enrichment (in single factor experiments, acclimation to, e.g., warming is tested for by comparing ambient and heated treatments at one common temperature). In our case, only the BR at growth conditions may give an impression of potential downregulation (i.e., lower R_{soil} under the future climate scenario than expected from measurements at ambient conditions), with homeostasis occurring if this BR is equal for both climate scenarios. Homeostasis would thus imply that climate scenario has no effect on R_{soil}. Therefore, we will only discuss differences in BR at growth conditions, that is, BR at 10°C for the ambient treatment versus BR at 13°C for the future climate scenario. The same accounts for the potential heterotrophic respiration. Therefore, we computed R_{hpot} at 23°C for the future climate scenario (for all treatments, R_{hpot} was measured at 20°C), to obtain R_{hpot} with a 3°C difference between both climate scenarios. To this end, we used a Q_{10} of two and computed R_{hpot} at 23°C for the simulated future climate scenario using (1) with b = 23°C.

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![Image of Soil respiration (R_{soil}) in function of soil temperature, for all replicates of each treatment. Dots are measured values; the line represents the regression fitted with (1).](image-url)
climate effects and AMF x climate scenario interactions, with chamber nested within climate scenario. Differences are reported significant at \( P \leq .10 \). When not mentioned, the interaction was not significant. In order to test whether AMF root colonization frequency and intensity in the inoculated pasteurized soil significantly differed between both climate scenarios, data were arcsin \( \sqrt{x/(x/100)} \) transformed before performing a one-way ANOVA.

### 3. Results

#### 3.1. AMF Root Colonization

In our experiment, both pasteurization and inoculation were successful. Throughout the season, only one replicate of the non-inoculated pasteurized soil showed minor AMF colonization (Zavalloni, unpublished). At the end of the season (November 2007), inoculation had resulted in 10% (SE = 3.6) and 20% (SE = 5.8) root colonization frequency and intensity, respectively, in the top 9 cm soil of the ambient treatment. Under warming and CO₂ enrichment, AMF root colonization frequency and intensity in the top 9 cm soil were on average 27.5% (SE = 6.3) and 30.7% (SE = 6.0), respectively. Differences in AMF root colonization frequency differed significantly between both climate scenarios (\( F_{1,6} = 5.75; P = .05 \); one-way ANOVA), whereas colonization intensity did not significantly differ between both treatments (\( F_{1,6} = 1.69; P = .24 \)).

#### 3.2. Root and Soil Characteristics

The entire profile root biomass revealed a significant AMF x climate scenario interaction, and a similar tendency was observed for root biomass and length of the top 9 cm soil (Table 1). Hence, the climate effect on root biomass differed between inoculated and non-inoculated pasteurized soil. Whereas we observed a tendency towards a negative climate effect in the inoculated pasteurized soil, root biomass, and root length in the non-inoculated pasteurized soil were higher under the future climate scenario (albeit not significantly).

Despite similar average root diameters across the treatments (Table 1), specific root length (SRL) showed a borderline significant AMF x climate scenario interaction effect (Table 1). Whereas SRL was slightly higher in IE than in NE (for the entire profile), SRL showed no difference between both soils at ambient conditions.

This pattern of AMF determining the climate response recurred in the root C : N ratio. Although neither root C or N concentrations nor root C : N ratio responded significantly to inoculation or warming and CO₂ enrichment (Table 1), we found a borderline significant AMF x climate scenario interaction for root N concentration and a significant AMF x climate scenario interaction for root C : N ratio (Table 1). Whereas the inoculated pasteurized soil exhibited a tendency towards a positive climate effect on root N concentration, we observed a slight decrease in NE as compared to NA.

### Table 1: Root and soil characteristics for the different treatments (mean and one standard error (SE)). Letters indicate significant differences within one row (\( P \leq .10 \); nested two-way ANOVA). Absence of letters denotes the absence of significant differences for the specific parameter. We also report (borderline) significant treatment (AMF or climate scenario) effects and AMF x climate scenario interactions.

<table>
<thead>
<tr>
<th></th>
<th>Inoculated pasteurized soil</th>
<th>Non-inoculated pasteurized soil</th>
<th>Statistic results for AMF x climate scenario interaction effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ambient climate</td>
<td>Future climate</td>
<td>Ambient climate</td>
</tr>
<tr>
<td>Top 9 cm</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>4.14</td>
<td>1.28</td>
<td>1.64</td>
</tr>
<tr>
<td>Entire profile</td>
<td>1.78b</td>
<td>0.47</td>
<td>1.22a</td>
</tr>
<tr>
<td>Root length (cm cm⁻³)</td>
<td>Top 9 cm</td>
<td>35.24</td>
<td>4.33</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>17.99</td>
<td>3.04</td>
<td>16.64</td>
</tr>
<tr>
<td></td>
<td>Top 9 cm</td>
<td>10.28</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>11.65</td>
<td>0.88</td>
<td>13.12</td>
</tr>
<tr>
<td></td>
<td>Top 9 cm</td>
<td>0.24</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>0.01</td>
<td>0.23</td>
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<tr>
<td></td>
<td>Top 9 cm</td>
<td>42.61</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
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<tr>
<td></td>
<td>4.14</td>
<td>1.28</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>1.78b</td>
<td>0.47</td>
<td>1.22a</td>
</tr>
<tr>
<td>Root N concentration (%)</td>
<td>Top 9 cm</td>
<td>0.93</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>46.17</td>
<td>0.90</td>
<td>40.84</td>
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<tr>
<td></td>
<td>1.32</td>
<td>0.05</td>
<td>1.33</td>
</tr>
<tr>
<td>Root C : N ratio</td>
<td>Top 9 cm</td>
<td>1.30</td>
<td>0.04</td>
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<td>SE</td>
<td>Mean</td>
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<tr>
<td></td>
<td>0.12</td>
<td>0.01</td>
<td>0.12</td>
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<tr>
<td></td>
<td>11.30</td>
<td>0.15</td>
<td>11.23</td>
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<tr>
<td>Soil C concentration (%)</td>
<td>Top 9 cm</td>
<td>0.12</td>
<td>0.00</td>
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<tr>
<td></td>
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<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>11.09</td>
<td>0.14</td>
<td>11.17</td>
</tr>
</tbody>
</table>

* Specific root length was calculated from root biomass and root length.
Figure 2: Mean potential heterotrophic respiration ($R_{\text{hpot}}$) measured at 20°C for the inoculated and non-inoculated pasteurized soil under ambient conditions (black bars) and under combined warming and CO$_2$ enrichment (grey bars). For the future climate scenario, we also computed $R_{\text{hpot}}$ at 23°C (white bars), using (1) with $b = 23°C$ and $Q_{10} = 2$, such that we could compare $R_{\text{hpot}}$ at growth conditions (for more details, see Section 2). Error bars represent the standard deviation on the mean.

Table 2: Weighted mean $Q_{10}$ (weighted using $1/(\text{SE of } Q_{10})$) and one standard error (SE) on WM for the different treatments. The $Q_{10}$ was calculated by fitting (1) to the data.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambient climate</th>
<th>Future climate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated pasteurized soil</td>
<td>2.10 0.13</td>
<td>2.12 0.13</td>
</tr>
<tr>
<td>Non-inoculated pasteurized soil</td>
<td>2.05 0.13</td>
<td>2.57 0.12</td>
</tr>
</tbody>
</table>

Effect: $F_{1,7} = 5.69; P = .05$). Differences between inoculated and non-inoculated pasteurized soil were not statistically significant.

3.4. Soil Respiration. In the inoculated pasteurized soil, basal soil respiration at growth conditions was similar in IA and IE (Figure 3), indicating a homeostasis of $R_{\text{soil}}$. The non-inoculated pasteurized soil revealed a trend towards an increased basal rate at growth conditions under the future climate scenario as compared to ambient conditions (Figure 3), but effects of climate scenario and AMF inoculation were not significant (weighted two-way ANOVA). The $Q_{10}$ of $R_{\text{soil}}$ varied around two in all treatments (Table 2); a weighted two-way ANOVA revealed no significant climate scenario or AMF effects.

4. Discussion

4.1. Root Characteristics. In our study, a joint rise in temperature and atmospheric CO$_2$ did not significantly alter root length or biomass. However, the significant AMF x climate scenario interaction effect implies that the climate effect differed between non-inoculated and inoculated pasteurized soil. The tendency towards a positive climate effect on root biomass in the non-inoculated pasteurized soil agrees with a previous study on the combined effect of warming and CO$_2$ enrichment [48] (but see [49]) and is probably primarily due to the elevated CO$_2$ concentrations. Whereas CO$_2$ enrichment usually enhances root growth and biomass [11, 50, 51], warming either decreases [52–54] or does not affect root biomass [49, 51, 55]. According to Fitter et al. [24], root growth is determined more by resource availability and source-sink relationships than by dominant environmental parameters such as temperature. This may clarify why in the non-inoculated soil root biomass tended to be higher at the future climate scenario than at ambient conditions, a response frequently ascribed to a CO$_2$-induced increase in carbon supply and belowground allocation, but usually not found under elevated temperatures.

In contrast to the non-inoculated pasteurized soil, root length and biomass in the inoculated pasteurized soil tended to be lower under elevated CO$_2$ and warming than under ambient conditions. We believe that this significantly different climate effect between inoculated and non-inoculated soil is related to the positive climate effect on AMF root colonization. It is well known that AMF act as a sink for carbon; in exchange for nutrients, carbon is transferred from

The opposite pattern was, logically, observed for root C : N ratio. Soil C and N concentrations were similar across all treatments (Table 1).

3.3. Potential Heterotrophic Respiration. For all treatments, $R_{\text{hpot}}$ decreased over time (Figure 2), reflecting the expected decrease of labile carbon during the incubation period. After six days of incubation, $R_{\text{hpot}}$ at growth conditions (black and white bars in Figure 2) was higher under the future climate scenario than under ambient conditions (significant scenario
the roots to the fungal symbiont. Furthermore, colonization by mycorrhizal fungi frequently resulted in reduced root biomass [56–58], although not all species showed such response [58, 59]. Hence, in the inoculated pasteurized soil, the extra carbon supply at the future climate scenario as compared to ambient conditions was likely transferred to the AMF, in turn reducing root biomass. This postulation is supported by measurements of photosynthesis and aboveground biomass. Whereas the future climate scenario stimulated photosynthetic rates, aboveground biomass was similar across all treatments (Zavalloni, unpublished). Hence, the extra carbon gained under warming and CO2 enrichment was not invested in aboveground biomass and was most likely transferred to AMF. In the non-inoculated pasteurized soil, carbon could not be transferred to a symbiotic partner, and plants could not rely on AMF for their nutrient supply. Consequently, plants in NE invested more carbon in their roots than plants in IE, which resulted in a significantly higher root biomass in NE as compared to IE.

4.2. Soil Respiration. Our study revealed no significant changes of $R_{\text{soil}}$ in response to warming and CO2 enrichment. The inoculated pasteurized soil even revealed a homeostasis of $R_{\text{soil}}$, as BR at growth conditions was nearly equal for both climate scenarios. This contrasts with most CO2 enrichment studies that revealed an increased $R_{\text{soil}}$ in response to elevated CO2 [14]. Warming also was frequently observed to enhance $R_{\text{soil}}$ [15], although thermal acclimation is often occurs (e.g., [60]). Responses of $R_{\text{soil}}$ to a joint rise in temperature and atmospheric CO2 have been rarely investigated (and most studies were performed on trees), and results of the few studies available are inconsistent [20, 22, 25, 26]. Wan et al. [26] demonstrated that factors such as soil moisture may be responsible for this variability. In our study, soil water content at the end of the growing season did not differ across the treatments (Zavalloni, unpublished). Hence, the observed homeostasis of $R_{\text{soil}}$ was unlikely due to water limitations.

The three components of $R_{\text{soil}}$, that is, root, mycorrhizal, and heterotrophic respiration may all alter in response to environmental changes and it is the combination of all three components that caused the homeostasis of $R_{\text{soil}}$. Given the positive climate effect on AMF root colonization, mycorrhizal respiration was likely higher in IE than in IA. Also heterotrophic respiration was probably higher in IE than in IA, as $R_{\text{bps}}$ responded positively to the simulated future climate scenario. Root respiration, the last component of $R_{\text{soil}}$, is determined by root biomass and by specific root respiration (i.e., respiration per unit of root biomass). Specific root respiration is often positively related to root N concentration. Because root N concentration did not significantly differ between IE and IA, we assume that the specific root respiration was similar for both treatments. Hence, decreased root respiration as a consequence of lower root biomass in IE than in IA (−30%, but statistically not significant) is the only component of $R_{\text{soil}}$ that can clarify the homeostasis observed in the inoculation pasteurized soil.

Similar to the inoculated soil, the non-inoculated pasteurized soil also did not exhibit a significant difference in $R_{\text{soil}}$ between both climate scenarios, suggesting a potential homeostasis. In contrast to the inoculated pasteurized soil, however, a homeostasis of $R_{\text{soil}}$ was unlikely in the non-inoculated pasteurized soil, as BR at growth conditions tended to be circa 20% higher in NE than in NA. Possibly, $R_{\text{soil}}$ was too heterogeneous to detect a significant difference between NE and NA with six replicates. In conclusion, we found no real indication for homeostasis of $R_{\text{soil}}$ in the non-inoculated pasteurized soil, in particular because both root biomass and $R_{\text{bps}}$ tended to increase in NE as compared to NA. This supports our statement that the positive climate effect on AMF root colonization in the inoculated pasteurized soil induced the homeostasis of $R_{\text{soil}}$ via a reduction in root biomass, as neither a homeostasis of $R_{\text{soil}}$, nor an AMF-induced reduction in root biomass was apparent in the non-inoculated pasteurized soil.

Both inoculated and non-inoculated pasteurized soil exhibited similar soil respiration rates. Apparently, AMF did not stimulate $R_{\text{soil}}$, as $R_{\text{bps}}$ was similar for AMF treatments. Furthermore, in the simulated future climate, root biomass was significantly lower in IE than in NE. This decrease was, however, not reflected in $R_{\text{soil}}$, which was similar for inoculated and non-inoculated pasteurized soil. Presumably, mycorrhizal respiration, which occurred only in IE, not in NE, and perhaps also specific root respiration (root N concentration was slightly higher in IE than in NE), compensated for the lower root biomass and associated root respiration in IE as compared to NE. Likewise, Cavagnaro et al. [30] concluded that effects of an AMF-induced decrease in root biomass and root length density on $R_{\text{soil}}$ were compensated by higher respiration rates of mycorrhizal roots per unit weight as compared to non-mycorrhizal roots.

Last, the temperature sensitivity of $R_{\text{soil}}$ was similar across all treatments. The lack of a climate effect on $Q_{10}$ is in agreement with Wan et al. [26], who observed no change in the $Q_{10}$ of $R_{\text{soil}}$ under warming and/or CO2 enrichment in wet conditions, but under dry conditions they observed a tendency towards a positive warming effect on $Q_{10}$ of $R_{\text{soil}}$ at both ambient and elevated CO2 [26]. The similar $Q_{10}$ in IE and IA gives further support to our statement that the homeostasis of $R_{\text{soil}}$ was primarily due to the lower root biomass in IE, as differences in substrate quality or substrate limitation would have been reflected in the $Q_{10}$ [61]. Further, we found no AMF effect on the $Q_{10}$ of $R_{\text{soil}}$, supporting earlier observations from Baath and Wallander [62] and Langlely et al. [29], who found no effect of AMF root colonization on the temperature sensitivity of root-derived respiration or soil respiration.

5. Conclusions

This study assessed the role of AMF in belowground carbon cycling under current and possible future climate conditions. Combined warming and CO2 enrichment led to increased AMF root colonization. AMF inoculation and the simulated future climate conditions interacted significantly in their effects on root biomass of artificial six-species grassland communities. Reduction of root biomass upon AMF inoculation resulted in a declined soil respiration.
response to a joint rise of temperature and atmospheric CO₂. This may suggest that AMF can contribute to an attenuated stimulation of Rₘₐₜ under a scenario of both rising atmospheric CO₂ and temperature.

Acknowledgments

This project was supported by the Belgian Science Policy-Program 'Science for a Sustainable Development' under contract MYCARBIO, no. SD/BD/05A. The authors thank Joke Van den Berge, Birgit Gielen, and especially Fred Kockelbergh for their valuable help during the set-up of the experiment. They thank Piergiorgio Stevanato for his kind assistance. Sara Vicca holds a grant from the Institute for the promotion of innovation through science and technology for the C and N analyses, and Stefan Van Dongen for statistical help. Nadine Calluy and Kristine Kockelbergh for their valuable help during the set-up of the experiment. They thank Piergiorgio Stevanato for his kind assistance. They also thank two anonymous reviewers and the subject editor for providing constructive comments on a previous version of the article. Last, I wish to thank Lola for being my lucky angel.

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