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**The role of wild boar (*Sus scrofa* L.) rooting
in forest ecosystems in Switzerland**

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Table of contents

Summary	1
<hr/>	
Zusammenfassung	5
<hr/>	
General introduction	9
<hr/>	
Paper I Effects of wild boar (<i>Sus scrofa</i> L.) rooting on hardwood forest soil carbon and nitrogen concentrations and pools in Switzerland	17
<hr/>	
Paper II Effects of wild boar (<i>Sus scrofa</i> L.) rooting on the bacterial community structure in mixed-hardwood forest soils in Switzerland	35
<hr/>	
Paper III Do changes in soil properties after rooting by wild boars (<i>Sus scrofa</i> L.) affect understory vegetation in Swiss hardwood forests?	55
<hr/>	
Paper IV Grubbing by wild boars (<i>Sus scrofa</i> L.) and its impact on hardwood forest soil carbon dioxide emission in Switzerland	73
<hr/>	
Synthesis	97
<hr/>	
Acknowledgements	101
<hr/>	
Curriculum vitae	103
<hr/>	

Summary

At the beginning of the 20th century, wild boars (*Sus scrofa* L.) were almost extinct in numerous European countries. Reduced to small, fragmented groups, wild boars were able to recover and re-colonize their former geographical range during the last decades, including their former habitat in Switzerland. Possible reasons for boars' increase in number and expansion in habitat range include their opportunistic feeding behaviour, high reproductive potential, and adaptability to a wide variety of habitats (ecological plasticity). In addition, expanded corn growing has come to be widely regarded as a reason for the continued increase in population sizes.

Corresponding to the dramatic growth of the boar population, reports of wild boar rooting in agricultural fields and forests have markedly increased. Whereas the economical damages in agriculture have been discussed intensively, the effects of boar rooting on forest ecosystems remain obscure. Although several studies have assessed the effects of wild boar rooting on individual forest soil and/or vegetation properties, or limited combinations of these properties, the impact of wild boars on the entire forest ecosystem processes remains unclear. The main goal of this study therefore was to obtain a better understanding of the effect of wild boar rooting on belowground (soil) and aboveground (vegetation) properties, and to gain insight into the complex interaction of these disturbance-sensitive forest properties.

The first part of this thesis was dedicated to assessing the effect of wild boar rooting on concentrations and pools of organic matter (OM), carbon (C) and nitrogen (N). Since wild boars incorporate the litter layer into the mineral soil while rooting, we expected that the turnover rates of C and N would be enhanced on rooted compared to non-rooted plots. Indeed, we found significantly higher concentrations of OM, C, and N in the mineral soil (0–15 cm and 15–30 cm) on rooted plots. Correspondingly, the concentrations of the litter layer were significantly lower on rooted plots. As expected, the soil bulk density was also affected by wild boar rooting with significantly lower values in the upper mineral soil horizons (0–15 cm) on rooted plots. Significant smaller OM, C, and N pools were found in the litter layer of rooted plots. In contrast, OM, C, and N pools in the mineral soil did not show any effect of rooting by wild boars. The counteracting combination of higher OM, C, and N concentrations and lower bulk density on rooted plots (and vice versa lower concentrations and higher bulk

densities on non-rooted plots) may make the net effect of rooting on soil pools difficult to detect.

Since bacteria are essential in edaphic processes such as nutrient cycling, decomposition or carbon storage, the second part of this thesis was dedicated to assessing whether wild boar rooting has an effect on the composition of soil bacterial communities. Our findings did not reveal any statistically significant difference in soil bacterial community composition or any community diversity indices between rooted and non-rooted plots. In contrast to the lack of a rooting effect, we found a clear seasonal pattern on bacterial community compositions, i.e., large differences between May, August and December. Assessing correlations between bacterial community compositions and various environmental parameters, namely plant available nitrogen, soil moisture and temperature, revealed several reasons for these seasonal differences.

Following the detailed assessment of soil parameters in the first two parts of this thesis, the third part focused on how rooting affects the understory vegetation of forest ecosystems. Will the detected soil property changes inevitably lead to changes in the vegetation? And will the wild boars' direct foraging effects on vegetation (uprooting, mechanical damage of various plant parts, consumption of plant seeds and shoots, etc.) increase or counteract the impacts on the vegetation through changes in soil properties? To answer these questions, we used data of soil and vegetation parameters measured over the course of four years. Increased microbial biomass suggested that the elevated carbon concentrations on the rooted plots served as resource for the decomposing organisms. However, instead of increasing the plant available N by increasing mineralization rates, the microbes rather seemed to immobilize N. We found lower amounts of plant available N on the rooted plots even though total N values were higher. Obviously, the relatively high C/N ratio of incorporated OM (leaf litter) forced the soil microbes to scavenge the soil solution to obtain enough N (which is required for the balance of nutrients). Consequently, more N was immobilized by the microbial community through incorporation into microbial cells, which depleted plant-available soil N. The rather detrimental (direct) effects of rooting on vegetation (e.g. mechanical damage, uprooting) resulted in reduced total plant cover and lower sapling counts on rooted plots. In contrast, height of saplings (< 1m) and plant species diversity did not differ between rooted and non-rooted plots. To study the impacts on the vegetation in more detail, additional, long-term assessments over the course of more than a couple of years or even decades would be useful, but would have gone beyond the scope of this study.

In the last (fourth) part of this study, we assessed the effect of wild boar rooting on forest soil carbon dioxide (CO₂) emissions. Elevated microbial biomass C and fine root biomass found on rooted plots were probably responsible for the elevated CO₂ emissions found on the rooted plots. Because up to 54% of the forest soil under study has been rooted by wild boars, and CO₂ emissions on the rooted plots are elevated by 20% or more compared to non-rooted plots, we conclude that at least on a regional scale, carbon cycling may be influenced by wild boars.

Summing up, wild boars appear to have a non-negligible impact on many aspects of forest ecosystems. Given the ongoing increase in the population of this prominent game species and the expansion of its habitat range in Switzerland, the knowledge gained in this study will be valuable for future game-forest-agriculture debates.

Zusammenfassung

Anfang des 20. Jahrhunderts waren Wildschweine (*Sus scrofa* L.) in vielen Gebieten Europas beinahe ausgerottet. Auf wenigen, isolierten Restvorkommen gründend, haben sich die Wildschwein-Populationen während den vergangenen Jahrzehnten jedoch in ganz Europa wieder stark vermehrt. Somit haben sich die Wildschweine weite Teile ihres ursprünglichen Verbreitungsgebiets zurückerobert und sind heute wieder grossflächig verbreitet, so auch in der Schweiz. Als mögliche Gründe für die wachsenden Bestände und die damit verbundene Ausbreitung gelten drei Hauptmerkmale des Wildschweins: opportunistisches Nahrungsspektrum, hohes Reproduktionspotential und eine hohe Anpassungsfähigkeit an eine Vielzahl verschiedener Habitats (ökologische Plastizität). Vielerorts wird auch der vermehrte Maisanbau der Landwirtschaft als Begründung beigezogen.

Entsprechend der stark erhöhten Wildschwein-Populationen haben sich Berichte über Wühlschäden in Forst- und vor allem Landwirtschaft gehäuft. Während die ökonomischen Folgen dieser Schäden ein grosses Diskussionsthema sind, ist über deren Einfluss auf Waldökosystemprozesse nur sehr wenig bekannt. Obwohl sich mehrere Autoren mit dem Einfluss von Wildschweinen auf einzelne Boden- oder Vegetations-Parameter im Wald beschäftigten, bleibt die Frage nach dem Einfluss dieser Wildart auf das Ökosystem Wald als Ganzes unbeantwortet. Das Gefüge einer Vielzahl von unterirdischen (Boden) und oberirdischen (Vegetation) mehr oder weniger störungsanfälliger Einzelparameter zu verstehen sowie deren Reaktion auf die durch die Wühltätigkeit der Wildschweine hervorgerufenen Störungen zu untersuchen, waren denn auch die Ziele dieser Arbeit.

Um den Einfluss von Bodenstörung durch die Wildschweine auf Waldökosystem-Prozesse zu ergründen, ist es unerlässlich die im Boden wichtigen Kohlenstoff- und Nährstoffkreisläufe zu verstehen. Im ersten Teil dieser Arbeit untersuchte ich folglich den Einfluss der Wildschwein-Wühltätigkeit auf die Konzentrationen und Pools von organischem Material (OM), Kohlenstoff (C), und des wichtigsten Pflanzen-Nährstoff im Boden, Stickstoff (N). Da Wildschweine beim Wühlen die organische Bodenaufgabe unter den Mineralboden mischen, ist zu erwarten, dass in gewühlten Flächen der Kohlenstoff- und Nährstoffumsatz im Vergleich zu ungewühlten Flächen erhöht ist. Entsprechend fanden wir signifikant höhere Konzentrationen von organischem Material, Kohlenstoff und Stickstoff im Mineralboden (0-

15 cm und 15-30cm) auf gewühlten Flächen. Die Werte in der Streuschicht waren parallel dazu signifikant tiefer auf den gewühlten Flächen. Wie erwartet, wurde durch die Wühltätigkeit auch die Bodendichte beeinflusst. Signifikant tiefere Werte wurden jedoch nur für die oberen Mineralboden-Horizonte (0-15cm) der gewühlten Flächen gefunden. Die Untersuchung der OM, C und N Pools zeigte signifikant tiefere Werte für die Streuschicht auf gewühlten Flächen, der Mineralboden zeigte indes keine Änderung. Die „antagonistische“ Kombination von höheren OM, C und N Konzentrationen und tieferen Bodendichten auf gestörten Flächen (und umgekehrt tiefere Konzentrationen und höhere Bodendichten auf ungestörten Flächen) liess den Wühleffekt auf die daraus errechneten Pools verschwinden.

Der zweite Teil dieser Arbeit konzentrierte sich auf die Frage, ob das Wühlen der Wildschweine Einfluss auf die Zusammensetzung der bakteriellen Gesellschaft im Boden nimmt, denn Bakterien sind in vielen edaphischen Prozessen wie Nährstoffkreislauf, Zersetzung, Kohlenstoffspeicherung usw. von zentraler Bedeutung. Wir konnten auf unseren Versuchsflächen keine Unterschiede in der Zusammensetzung der bakteriellen Gesellschaft oder verschiedenen Diversitäts-Indices zwischen gewühlten und ungewühlten Flächen finden. Im Gegensatz zum fehlenden Wühleffekt, fanden wir jedoch saisonale Unterschiede in der Zusammensetzung der bakteriellen Gesellschaft. Dank Korrelationen zu verschiedenen Umweltparametern, namentlich pflanzenverfügbarer Stickstoff, Bodenfeuchtigkeit und Bodentemperatur, konnten die Gründe für diese Unterschiede eruiert werden. Offensichtlich überdeckten die saisonalen Unterschiede den Effekt der Bodenstörung durch die Wildschweine.

Nach der detaillierten Untersuchung von verschiedenen Bodenparametern in den zwei ersten Teilen dieser Arbeit, galt das Hauptaugenmerk des dritten Teils der Vegetationsschicht im Wald. Führen die festgestellten Veränderungen der Bodenparameter unweigerlich auch zu Veränderungen der Vegetation? Und verstärken die direkten Einflüsse der Wildschweine auf die Vegetation (Entwurzeln, mechanische Schädigung von verschiedenen Pflanzenteilen, Fressen von Pflanzensamen, -trieben usw.) die Auswirkungen der veränderten Bodenparameter zusätzlich oder wirken sie diesen entgegen? Um diesen und ähnlichen Fragen auf den Grund zu gehen wurden verschiedene Boden- und Vegetationsparameter über die Dauer von 4 Jahren gemessen. Die höheren Kohlenstoffwerte auf den gewühlten Flächen dienten den zersetzenden Organismen als Nahrungsquelle, welche diese mit einer höheren Biomasse quittierten. Obwohl die Stickstoff-Konzentration im Mineralboden ebenfalls erhöht

war, wurde die Menge des pflanzenverfügbaren Stickstoffs durch das Wühlen nicht etwa erhöht, sondern reduziert. Offensichtlich wurde der Stickstoff grösstenteils von den zersetzenden Organismen immobilisiert und die Menge des pflanzenverfügbaren Stickstoff dadurch reduziert. Die eher „pflanzenschädlichen“ direkten Einflüsse (mechanische Schädigung, Entwurzelung) sind wohl verantwortlich für die reduzierte Pflanzen-Bedeckung sowie die tiefere Anzahl der Jungbäume. Hingegen konnten keine Unterschiede betreffend der Höhe von Jungbäumen (< 1m) sowie der Pflanzen-Diversität zwischen gewühlten und ungewühlten Flächen gefunden werden. Um die Auswirkungen betreffend Vegetation genauer zu untersuchen, bräuchte es zusätzliche, längerfristige Untersuchungen über mehrere Jahre (Jahrzehnte), was jedoch den Rahmen dieser Arbeit sprengt.

Im vierten und letzten Teil dieser Arbeit wurde der Einfluss der Wühltätigkeit von Wildschweinen auf den Kohlendioxidausstoss (CO_2) des Waldbodens untersucht. Die auf den gewühlten Flächen gefundenen höheren Werte der mikrobiellen Biomasse sowie der Feinwurzel-Biomasse waren wahrscheinlich verantwortlich für den ebenfalls gesteigerten CO_2 -Ausstoss, der auf den gewühlten Flächen resultierte. Der um mehr als 20% erhöhte CO_2 -Ausstoss und die Tatsache, dass an unseren Untersuchungsstandorten bis zu 54% des Waldbodens durch Wildschweine aufgewühlt wurde, lässt zumindest auf lokaler Ebene auf eine Beeinflussung des Kohlenstoffkreislaufes durch Wildschweine schliessen.

Zusammenfassend scheinen die Wildschweine einen nicht zu vernachlässigenden Einfluss auf zahlreiche Parameter des Ökosystems Wald zu haben. Die noch immer anhaltende Zunahme und Ausbreitung des Wildschweins in der Schweiz, unterstreicht zudem wie wichtig die Erkenntnisse dieser Arbeit über diese markante und charakteristische Wildart in der Schweiz für zukünftige Kulturland-Wald-Wild Diskussionen sein dürften.

General introduction

Wild boars (*Sus scrofa* L.) considerably increased in population sizes and expanded their ranges in Switzerland during the past two decades as in many other European countries (Boitani et al. 1995; Schley et al. 1998; Geisser and Reyer 2005; Hebeisen et al. 2008). The most likely reasons for the widespread expansion of these animals are opportunistic feeding behavior, high reproductive potential and high adaptability to a wide variety of habitats (high ecological plasticity; Boitani et al. 1995; Taylor et al. 1998). Wild boars are omnivorous and obtain a considerable proportion of their diet by rooting (grubbing) in the soil searching for plant seeds, roots/bulbs and vertebrate and invertebrate animals. (e.g. Howe and Bratton 1976; Wood and Roark 1980; Bratton et al. 1982; Baber and Coblenz 1987; Hone 1988). Rooting involves breaking through the surface layer of the vegetation followed by excavation of the detected food item, thereby disturbing up to 80 % of the soil surface (Genov 1981, Howe et al. 1981, Risch et al. 2010). This excavation may be very superficial affecting only the litter layer, but typically impacts the top 15 cm of the organic and mineral soil layers (Genov 1981; Kotanen 1995). According to the increases in population sizes and ranges, reports of wild boar rooting events in agricultural and forest fields became more frequent, also in Switzerland. Whereas the economical damage of these activities in agricultural fields have been discussed intensively (e.g. Mackin 1970; Dardaillon 1986; Choquenot 1998; Schley and Roper 2003; Geisser and Reyer 2004; Wilson 2004; Seward et al. 2004) only few studies assessed the effect of this natural disturbance (bioturbation) on forest ecosystem processes. However, boar rooting may have a significant impact on carbon and nutrient cycling of forest ecosystems since major stores of nutrients and carbon are stored in the soil organic layers and upper mineral soil horizons in temperate forests. By mixing soil horizons and incorporating energy rich organic material into the mineral soil boar rooting can stimulate microbial growth and activity (Mallik and Hu 1997) and therefore enhance decomposition and mineralization rates (Lacki and Lancia 1983; Singer et al. 1984; Groot Bruinderink and Hazebroek 1996; Moody and Jones 2000; Mohr and Topp 2001; Mohr et al. 2005). These alterations in soil carbon and nutrient cycling can consequently affect understory vegetation growth and composition. In addition, boar rooting may also directly (adversely) affect understory vegetation properties through foraging (seed predation), displacing and killing seedlings, uprooting or mechanical damage of plants.

However, most authors that investigated boar rooting in forested ecosystems focused on single or few soil or vegetation parameters, and little attention has been given on how rooting affects interacting parameters or complex ecosystem processes. The present study aims to assess how rooting by boars affect different soil and vegetation properties in mixed hardwood forests, the forest type that is most frequently used as boar habitat in Switzerland. Each chapter of this thesis focused on several parameters, the overall synthesis provided at the end of this thesis (in the chapter “Synthesis”) will provide a comprehensive overview about the effect wild boar rooting has on Swiss forest ecosystems.

Objectives

The four main objectives of this thesis were:

- I) To investigate the effect of wild boar rooting on carbon and nutrient cycling, namely to assess whether C and N concentrations and pools of the forest floor and the mineral soil differ between rooted and non-rooted plots (Paper I).
- II) To investigate whether bacterial community structure of the forest soils showed any reaction to rooting (Paper II).
- III) To investigate if changes in soil properties after rooting affected the understory vegetation (Paper III).
- IV) To assess how rooting impacts forest soil carbon dioxide emissions (Paper IV).

Paper I: Effects of wild boar (*Sus scrofa* L.) rooting on hardwood forest soil carbon and nitrogen concentrations and pools in Switzerland

Carbon and nutrient cycling is crucial for numerous forest ecosystem processes as forest soils in Europe store roughly 1.5 times more C than trees (Baritz et al. 2010). Understanding soil C dynamics and the potential reaction on various disturbances therefore is of great interest. Forest ecosystem C and nutrient cycling is the result of interaction between climate, plant species, numerous abiotic and biotic soil properties and disturbance regimes (Bhatti et al. 2002). Thus, any alteration of one or several of those parameters can substantially influence other parameters and/or the complex system of C and nutrient cycling in forests. Through incorporation of litter into the mineral soil and mixing of soil layers, boar rooting may influence decomposition and therefore C and N cycling. In a first step, we therefore assessed potential effects of wild boar rooting on soil C and N concentrations. In a second step, we calculated soil C and N pools by taking into account soil bulk density, which is also supposed to be affected by boar rooting (Singer et al. 1984).

Paper II: Effects of wild boar (*Sus scrofa* L.) rooting on the bacterial community structure in mixed-hardwood forest soils in Switzerland

Soil bacterial communities play an important role in decomposition and nutrient cycling within forest soils (Cookson et al. 2008). Further, soil bacterial communities may act as early indicators of changes in soil quality as they respond rapidly to soil disturbance. Any disturbance of the soil physical integrity may have profound consequences on the bacterial community structure. In practice, however, microbial community responses in forest ecosystems range from low resilience, to high resilience, of even complete tolerance (Busse et al. 2006). Whereas numerous studies focused on the effects of forest management practices on soil bacterial communities, almost nothing is known about the effects of biotic natural disturbances, especially the ones caused by large animal. We therefore determined bacterial community structure as well as microbial biomass C on paired rooted and non-rooted study sites in four hardwood forest stands. We also measured soil moisture, soil temperature, and plant available inorganic nitrogen and correlated these data with the bacterial community structure of different sampling dates to assess how these parameters affected the bacterial community structure.

Paper III: Do changes in soil properties after rooting by wild boars (*Sus scrofa* L.) affect understory vegetation in Swiss hardwood forests?

After assessing possible effects of wild boar rooting on various soil properties, we also tried to understand how these changes affected the growth and composition of the understory vegetation within the forest stands under study. Beside the potential indirect effect of wild boar rooting on understory plants, wild boars may also influence the vegetation directly by foraging, uprooting or mechanical damage of plants. For this purpose, we measured soil chemical (C and N concentrations, and N availability) and biological (microbial biomass C) properties, as well as total plant cover, plant species diversity, and number and height of saplings on paired rooted and non-rooted plots in six hardwood forest stands in Switzerland. The corresponding results will show if positive (e.g. through higher nutrient availability) or negative effects (e.g. through mechanical disturbance or uprooting) of wild boar rooting on the understory vegetation will predominate or if the effects cancel each other and no effect of wild boar rooting is detectable on the understory vegetation at least at our study sites.

Paper IV: Grubbing by wild boars (*Sus scrofa* L.) and its impact on hardwood forest soil carbon dioxide emission in Switzerland *)

Carbon cycling is not only crucial for the forest ecosystem itself, but gained lots of attention regarding the sink/source discussion in global warming debates. CO₂ emissions undoubtedly play an important role in the terrestrial greenhouse gas balance. Understanding soil C dynamics and the potential reaction of these dynamics to various disturbances therefore is crucial. We are not aware of any study that would have investigated the effect of boar grubbing on soil CO₂ emissions. However, we expect the addition of energy rich organic material to stimulate microbial growth and activity and therefore an increase in CO₂ production (decomposition). To test this hypothesis, we measured CO₂ emission rates on paired grubbed and non-grubbed plots for a 3-year period. In addition we assessed soil moisture, and temperature, as well as fine root and microbial biomass, and correlated these data to soil CO₂ emissions. Finally, we modeled the total amount of CO₂ released from Swiss forest soils (large scale extrapolation) using grubbing frequency according to forest type and wild boar hunting statistics.

Significance of the project

We expect that the results of this thesis will significantly improve our knowledge of how a natural disturbance (wild boar) might alter soil physical, chemical, and biological parameters as well as vegetation properties in forested ecosystems. Consequently, this study will facilitate our understanding of animal-soil, animal-plant, and plant-soil interactions in forest ecosystems, which in turn will be needed to answer question related to ecosystem stability/resilience, biodiversity, and sustainable management. Further, the knowledge gained in this study will be of great importance for predictions on how ecosystem characteristics or processes might be altered when the abundance or spatial distribution of the wild boars changes in Switzerland. Consequently, the results of this study will be of great interest for both the research fields of animal and vegetation ecology as well as for conservation biology and forest sciences.

*) Grubbing in this chapter has the same meaning as rooting in the other chapters. Since the paper has already been published, we did not replace grubbing by rooting.

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Paper I

Effects of wild boar (*Sus scrofa* L.) rooting on hardwood forest soil carbon and nitrogen concentrations and pools in Switzerland

Submitted as:

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Abstract

Understanding soil carbon and nutrient cycling is important for assessing the effect of soil disturbances on forest ecosystem processes. Although numerous studies have evaluated anthropogenic or abiotic effects (e.g., forest management practices, fire) on forest soil OM, C, and N, concentrations and pools, considerably less information is available on the role of natural disturbance by large mammals. We found that rooting by wild boars (*Sus scrofa* L.) led to significant decreases of OM, C and N concentrations in the litter layer, and significant increases in the 0-15 cm and 15-30 cm (with exception of OM for 15-30 cm) mineral soil depth in 11 mixed hardwood stands in northern Switzerland. In addition, we found soil bulk density to be significantly lower on rooted compared to non-rooted plots for the top 15 cm of the mineral soil, but detected no difference for the 15 to 30 cm soil depth. Forest litter layer OM (42%), C (40%) and N (50%) pools were significantly smaller after rooting, but the mineral soil (0-30 cm) OM, C, and N pools were unaffected. Summing the changes across each profile reveals little net effect of rooting on C and N pools. Thus, we infer that rooting by boars redistributes C and N in soil profiles but does not alter the forest soil C/N sink-source dynamics within our stands. However, the incorporation of litter into the mineral soil and the concomitant decrease in soil bulk density could, over the longer time frames, lead to improved growth conditions for trees, grasses, forbs and microbes and therefore lead to long-term changes in C and N dynamics within the stands. Since roughly 410 km² of Swiss hardwood and mixed hardwood forests have boar densities comparable to our stands, boars

can be assumed to have a much larger impact on forest ecosystems than just the stand level. The impact of boars is expected to grow in the near future as they increase their populations and expand their ranges within Switzerland.

Keywords

Bioturbation, rooting, soil nutrient, organic matter, temperate forest

Introduction

Forest soils store approximately 40 percent of the world's below-ground terrestrial carbon (C; e.g., Dixon et al., 1994; Bhatti et al., 2002) and therefore play an important role in the global C cycle (e.g., Tans et al., 1990; Lal, 2005; Taylor et al., 2008). Consequently, interest in understanding the factors that affect these pools is high. The amount of C and N that a forest soil stores or releases is strongly related to its organic matter (OM) quality, as well as its micro-climatic (temperature, moisture), physical (texture, bulk density), chemical (pH, nutrient concentrations), and biological (microbial biomass, composition, diversity, activity) properties (e.g., Kirschbaum, 1995; Lomander et al., 1998; Epstein et al., 2002). Thus, any alteration of these parameters can increase or decrease rates of mineral soil OM decomposition and other forest soil processes, and therefore alter the total amount of C and N stored in the soil.

Various studies have shown that anthropogenic alterations of soil properties as a consequence of land use change or forest management can affect the storage and distribution of C and nutrients in soils (e.g., Mann, 1986; Johnson, 1992; Dixon et al., 1994; Kimmins, 1996; Lal, 2005; Page-Dumroese and Jurgensen, 2006; Taylor et al., 2008; Chertov et al., 2009). Similarly, abiotic disturbance factors such as wildfire (e.g., Bhatti et al., 2002; Bormann et al., 2008; Chertov et al., 2009) or wind throw (e.g., Liechty et al., 1997; Kramer et al., 2004; Thürig et al., 2005) can strongly impact soil C and N pools. In contrast, considerably less is known about the role of biotic disturbance in the C and nutrient dynamics of forest soils. Invertebrates have received some limited attention in the literature (e.g. Hunter et al., 2003; Risch et al., 2005; Kilpeläinen et al., 2007; Morehouse et al., 2008), while the impact of large mammals on forest soil C and nutrient processes has largely been neglected. We are aware of only one study that investigated the effect of large mammals on soil properties in forested ecosystems: Pastor et al. (1993) measured greater pools of potentially mineralizable carbon in boreal forest soils on Isle Royale, Michigan, USA where moose were excluded, compared to areas where they were allowed to browse. This lack of knowledge about how large mammals

impact forest soil C and nutrient dynamics is surprising, since large mammals are ubiquitous in most forested ecosystems (Mohr et al., 2005) and therefore could have large effects on forest soil C and nutrient concentrations and pools.

For example, the omnivorous wild boar (*Sus scrofa* L.) – an animal that obtains a considerable proportion of its diet by rooting (grubbing) in the soil searching for plant seeds, roots/bulbs, vertebrates and invertebrate animals (Howe and Bratton, 1976; Wood and Roark, 1980; Bratton et al., 1982; Baber and Coblenz, 1987; Hone, 1988) – acts as an extensive agent of natural disturbance in forest ecosystems. Rooting involves breaking through the vegetation, litter layer, and surface fine roots, and typically impacts the top 15 cm to 70 cm of the organic and mineral soil (Imeson, 1977; Genov, 1981; Kotanen, 1995). In forested ecosystems inhabited by wild boars, between 13 and 80 % of the soil surface can be rooted (Genov, 1981; Howe et al., 1981; Risch et al., 2010), and it has been shown that rooting enhances decomposition and mineralization rates (Lacki and Lancia, 1983; Singer et al., 1984; Groot Bruinderink and Hazebroek, 1996; Moody and Jones, 2000; Mohr and Topp, 2001; Mohr et al., 2005). However, most of these studies focused on ecosystems where non-native wild boars (feral pigs) had been introduced, and only very little is known of the ecological role wild boars may have on ecosystem where they are native. Further, we could not find a study that investigated how rooting affects forest soil C and nutrient pools.

The number of European wild boars has increased considerably during the past two decades (Apollonio et al. 2010) due to their opportunistic feeding behavior, high reproductive potential, and high adaptability to a wide variety of habitats (Boitani et al., 1995; Taylor et al., 1998). In Switzerland, population densities of wild boars are among the highest in Western Europe (Melis et al., 2006) reaching approximately 10 individuals/km² in some regions (Hebeisen et al., 2008). Thus, as a result of the ongoing increases in wild boar population densities and the continuous expansion of their geographical distribution, it is possible that wild boars could have a considerable impact on Swiss forest soil OM, C, and N dynamics. The objective of our study was, consequently, to assess how rooting by boars alters litter layer and mineral soil (to a depth of 30 cm) OM, C, and N concentrations and pools in mixed-hardwood forests, the forest type that is most frequently used as boar habitat in Switzerland. We also discuss the effect wild boars might have on C and N pools at the national scale.

Materials and Methods

Study area and sampling design

The study was conducted in eleven mixed hardwood stands in northern Switzerland. These stands were dominated by beech (*Fagus sylvatica* L.) but also contained other hardwoods [including oaks (*Quercus* ssp.) and hornbeam (*Carpinus betulus* L.)] and conifers [including Norway spruce (*Picea abies* L. Karst) and Scots pine (*Pinus sylvestris* L.)]. All sites were classified as typical *Galio oderati-Fagetum* following Keller et al. (1998) and were located within 55 km of each other. The closest distance between two sites exceeded 1 km. The elevations ranged from 540 to 850 m a.s.l. All sites were located on sandy loam or sandy clay loam soils, with soil pH ranging between 2.7 and 6.9 (Table 1). Each forest stand was inhabited by wild boars throughout the year and rooting was ubiquitous. We established a paired plot design consisting of a rooted (disturbed) and non-rooted (undisturbed) plot in each of the eleven stands in early spring of 2008. Both plots were 10 x 10 meters in size and were fenced with 1.3 m high knotted mesh (Ursus 130/11/15; mesh size at 0-50 cm height = 10 x 15 cm; mesh size at 50-110 cm = 15 x 15 cm; mesh size at 110-130 cm = 20 x 15 cm; Hortima AG, Hausen, Switzerland) tightened around 1.6 m square wooden posts (15 x 15 cm) to prevent boars (and roe deer) from entering. Two 2.5 mm diameter wires were affixed at 1.4 and 1.5 m height to prevent animals from jumping over the 1.3 m mesh.

Table 1. Site and soil characteristics of the top 30 cm of the mineral soil for the 4 sites under study.

Site	Elevation (m)	pH	Available water (g / 100 g soil)	Rock content (%)	Soil particle size distribution			Soil textural class
					Sand (%)	Silt (%)	Clay (%)	
1	540	3.4	11.9	55.8	74.7	15.0	10.3	Sandy loam
2	550	3.2	10.7	39.4	72.4	17.7	9.9	Sandy loam
3	560	2.9	18.1	28.4	74.5	16.7	8.9	Sandy loam
4	620	2.8	19.0	43.4	70.6	21.5	8.0	Sandy loam
5	660	2.8	20.2	22.9	66.8	24.3	8.9	Sandy loam
6	670	2.7	10.6	59.7	68.9	21.9	9.3	Sandy loam
7	570	4.1	17.1	0.0	63.8	12.1	24.1	Sandy clay loam
8	590	4.0	16.0	0.0	64.4	14.2	21.5	Sandy clay loam
9	610	4.3	17.6	0.0	69.1	16.0	15.0	Sandy loam
10	730	5.5	17.0	25.7	70.4	12.8	16.9	Sandy loam
11	850	6.9	23.1	20.4	70.3	14.3	15.4	Sandy loam

Since rooting generally is visible for roughly three years in the forest types we worked in, our non-rooted plots had not been disturbed for three years. To determine if boars specifically select foraging sites within a homogeneous stand or randomly root in the soil, we established a third randomly chosen, non-rooted and non-fenced 10 x 10 m plot at each study site at the beginning of the study (May 2006). All these plots were subject to rooting until the end of the study and, therefore, we assume that boars do not specifically select the sites where they are rooting. Thus, our randomly chosen non-rooted fenced plots were sufficient controls to be compared with the respective rooted plots. However, we cannot rule out that some of the differences in soil properties that we measured already existed prior to rooting. Unfortunately, such differences could not be accounted for as it is not possible to predict where the boars are going to root and therefore “pre-treatment” sampling was not possible (see also Risch et al. 2010).

Soil sampling and analyses

We randomly collected three circular litter layer samples (700 cm², with all surface organic horizons combined) in each rooted and non-rooted plot. One mineral soil core (5 cm diameter x 15 cm depth) was taken with a soil corer (AMS, American Falls, ID, USA) from each of the three locations where the litter layer was removed. The second soil core was taken from directly beneath the first, to a depth of 30 cm. Mineral soil sampling was performed at fixed depths since no clear soil horizon delineation was possible after rooting. All litter layer samples were oven dried at 65 °C (for 48 h) and ground to pass a 0.5 mm sieve. Mineral soil samples were oven dried at 65 °C (for 48 h), passed through a 2 mm sieve, and finely ground. Total N and C concentrations of all mineral and organic soil samples were analyzed on a LECO induction furnace at 950 °C (LECO Corporation, St. Joseph, Michigan, USA). Soil bulk density was estimated with the polyurethane foam technique (Page-Dumroese et al., 1999) to avoid soil compaction by using core sampling on the rooted plots. For this purpose, we carefully excavated mineral soil for both the 0 to 15 cm and 15 to 30 cm depth on a 10 x 10 cm area in rooted and non-rooted soils in each stand and filled the holes with polyurethane expanding insulation foam to determine the volume of the excavated soil. The soil was dried at 105 °C. Hole volume was determined by water displacement of the foam core after it was removed from the soil. Rock-fragments larger than 2 mm were sieved from the soil and weighed (Table 1). Total bulk density (ρ_{bT}) was calculated by dividing the oven-dry mass by the sample volume. Fine fraction soil bulk density (ρ_{bs}) was calculated with equation (1):

$$\rho_{bs} = \rho_{bT}(1 - g_r)/(1 - v_r) \quad (1)$$

where g_r (gravimetric rock content) was calculated by dividing the mass of the rock fragments by the total sample mass (Andraski 1991). Volumetric rock-fragment content (v_r) was calculated with formula (2):

$$v_r = \rho_{br}(g_r/\rho_{br}) \quad (2)$$

where the average rock-fragment density (ρ_{br}) was assumed to be 2.65 Mg m^{-3} . Organic matter (OM) content of the litter layer and mineral soil were determined by loss-on-ignition at 425°C for 16 h (Ben-Dor and Banin, 1989). Soil particle size distribution was estimated using the hydrometer method (Gee and Bauder, 1986). Soil pH was conducted on a 2:1 water to soil paste (Table 1). Available water capacity (AWC) was calculated after determining permanent wilting point (PWP) and field capacity (FC) using the pressure plate method (Klute, 1986).

Calculations and statistical analyses

We calculated litter layer and mineral soil (0-15 cm and 15-30 cm soil depth) C, N, OM concentrations and C:N-ratios by averaging the values obtained from the three samples collected at each plot. Litter layer C, N and OM pools (Mg ha^{-1}) were calculated using litter layer dry weight (g), sampling area (m^2), and C, N, and OM contents (g g^{-1} dry soil). Mineral soil C, N and OM pools (Mg ha^{-1}) were calculated using fine fraction bulk density (kg m^{-3}), sampling depth (m) and C, N and OM concentrations (g g^{-1} dry soil) for both the 0-15 cm and 15-30 cm soil depth separately (Homann et al., 1995; Wairiu and Lal, 2003). The effect of wild boar rooting on soil bulk density, litter layer and mineral soil C, N and OM concentrations, C:N ratios as well as C, N and OM pools was analyzed using Wilcoxon signed-rank tests for paired samples (performed with the R statistical package: Version 2.4.1, R, Development Core Team, 2006). The alpha level for all statistical tests was 0.05.

Results

Effect of rooting on OM, C, N concentrations and C:N ratios

Litter layer OM, C, and N concentrations were significantly lower on the rooted compared to the non-rooted plots (Figure 1a - 1c; Table 2A), decreasing by 6.8%, 7.1%, and 16.7%, respectively. However, no rooting effect on the C:N ratio could be found. The soil mixing activities of the boars resulted in significantly higher mineral soil OM (32.0%), C (29.9%), and N (25.0%) concentrations of the 0-15 cm depth on rooted compared to non-rooted plots (Figure 1d - 1f; Table 2B). The C:N ratio remained unaltered. We also found significantly higher C (24.1%) and N (100%) concentrations for the 15 to 30 cm soil depth on the rooted

compared to the non-rooted plots (Figure 1g - 1i; Table 2C), but again, the C:N ratio was not affected.

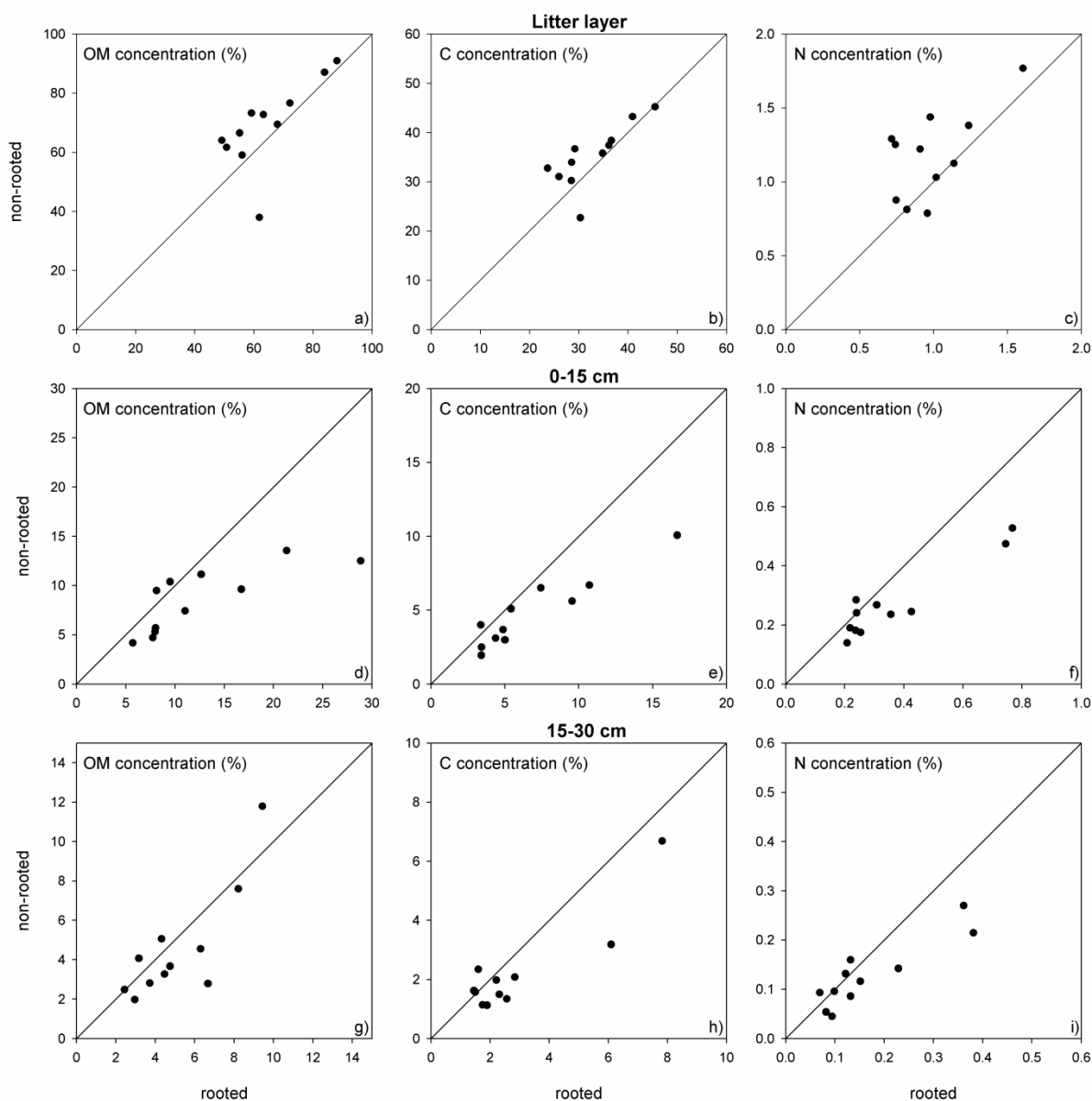


Figure 1. Comparison of soil OM, C, and N concentrations between rooted and non- rooted plots; a) – c): litter layer; d) – f): 0-15cm mineral soil depth; g) –i): 15-30 cm mineral soil depth. n = 11, for means and statistics see Table 2.

Table 2. OM, C and N concentrations, and C:N ratios of the (A) litter layer, (B) 0-15 cm mineral soil depth, and (C) 15 – 30 cm mineral soil depth on rooted and non-rooted plots. Significant differences between the rooted and non-rooted plots are indicated in bold. Values in parentheses are standard errors. n = 11, alpha-level = 0.05.

	OM conc. (%)	C conc. (%)	N conc. (%)	C:N ratio
(A) Litter layer				
grubbed	64.3 (3.8)	32.7 (2.0)	1.0 (0.1)	33.7 (1.3)
non-grubbed	69.0 (4.3)	35.2 (1.9)	1.2 (0.1)	30.7 (1.6)
P ^a	0.05	0.05	0.05	0.10
V ^b	11	11	11	52
(B) 0-15 cm				
grubbed	12.5 (2.1)	6.70 (1.2)	0.4 (0.1)	18.6 (1.3)
non-grubbed	8.5 (1.0)	4.70 (0.7)	0.3 (0.0)	17.6 (1.3)
P ^a	< 0.01	< 0.01	0.01	0.10
V ^b	63	64	61	52
(C) 15-30 cm				
grubbed	5.1 (0.7)	2.90 (0.6)	0.2 (0.0)	17.9 (1.7)
non-grubbed	4.5 (0.9)	2.20 (0.5)	0.1 (0.0)	18.7 (2.4)
P ^a	0.21	0.02	0.03	0.83
V ^b	48	58	57	30

^a P values determined by Wilcoxon signed-rank tests.

^b V values determined by Wilcoxon signed-rank tests.

Effect of rooting on soil bulk density and OM, C, and N pools

Rooting by wild boars significantly decreased litter layer OM (decreased by 41.9%), C (39.2%), and N (50%) pools (Figure 2a - 2c; Table 3A). As we would have expected, rooting also resulted in a significant decrease (33.5%) in fine-fraction bulk density of the 0-15 cm mineral soil (Table 3B). However, we found no differences in the OM, C, and N pools between the rooted and non-rooted plots, despite the higher OM, C and N concentrations detected (Figure 2d - 2f; Table 3B). These findings indicate that the increases in concentrations and decreases in bulk density were counteracting one another when pools are considered. For the 15 to 30 cm soil depth we could not detect any differences in soil bulk

density, OM, C or N pools between the rooted and non-rooted plots (Figure 2g - 2i; Table 3C).

We did not detect significant relationships between site-specific parameters such as soil pH, soil texture, permanent wilting point (PWP), available water capacity (AWC) or field capacity (FC) (Table 1) and OM, C, N concentrations or C:N ratios for any of the three soil layers. The same holds true for the relationship between the above mentioned parameters and OM, C and N pools.

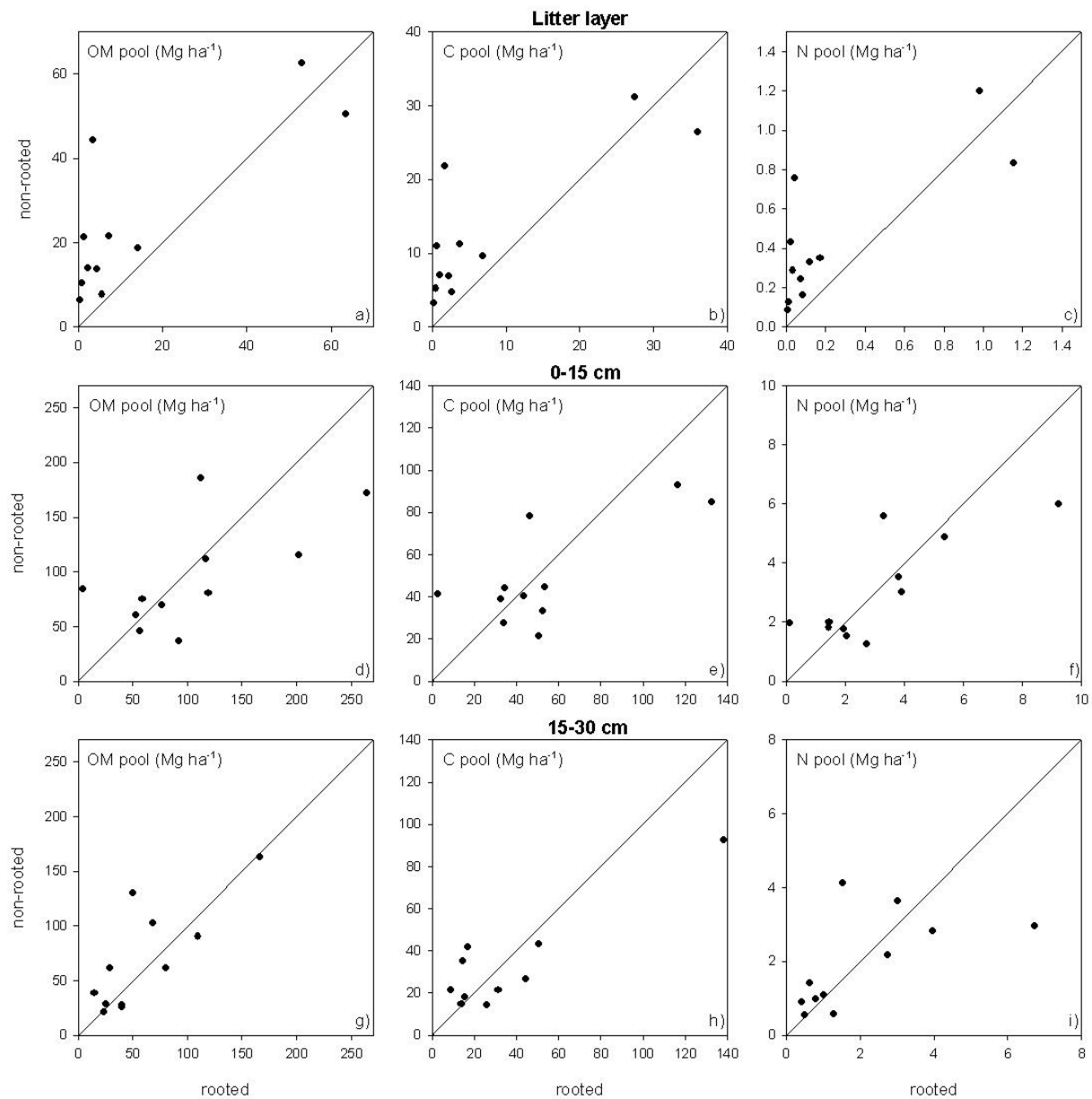


Figure 2. Comparison of soil OM, C, and N pools between rooted and non- rooted plots; a) – c): litter layer; d) – f): 0-15cm mineral soil depth; g) –i): 15-30 cm mineral soil depth. n = 11, for means and statistics see Table 3.

Table 3. Fine fraction bulk density, and OM, C and N pools of the (A) litter layer, (B) 0-15 cm mineral soil depth, and (C) 15 – 30 cm mineral soil depth on rooted and non-rooted plots. Significant differences between the rooted and non-rooted plots are indicated in bold. Values in parentheses are standard errors. n = 11, alpha-level = 0.05.

	Fine fraction bulk density (kg/m ³)		OM pool (Mg/ha)	C pool (Mg/ha)	N pool (Mg/ha)
(A) Litter layer					
grubbed	-	-	14.3 (6.7)	7.6 (3.7)	0.2 (0.1)
non-grubbed	-	-	24.6 (5.7)	12.5 (2.9)	0.4 (0.1)
P ^a	-	-	0.02	0.03	0.03
V ^b	-	-	8	9	9
(B) 0-15 cm					
grubbed	712.4 (89.5)		105.1 (22.1)	54.50 (11.4)	3.2 (0.7)
non-grubbed	1070.8 (76.0)		94.1 (14.5)	49.70 (7.3)	3.0 (0.5)
P ^a	< 0.01		0.46	0.64	0.70
V ^b	0		42	39	38
(C) 15-30 cm					
grubbed	1171.3 (105.3)		58.9 (13.7)	34.10 (11.2)	2.1 (0.6)
non-grubbed	1351.6 (45.6)		68.1 (14.4)	31.30 (6.9)	1.9 (0.4)
P ^a	0.15		0.58	0.97	0.97
V ^b	16		26	34	32

^a P values determined by Wilcoxon signed-rank tests.

^b V values determined by Wilcoxon signed-rank tests.

Discussion

Rooting effects on soil OM, C, and N concentrations

Rooting activities by boars resulted in decreased OM, C and N concentrations in the litter layer, but increased values in the mineral soil (with the exception of the OM concentration in the 15 to 30 cm soil layer). Rooting is known to incorporate the litter into the soil (Singer et al. 1984), leading to increased OM, C and N concentrations in the mineral layer. Simultaneously, rooting contaminates the remaining litter layer with dust and small mineral soil particles, leading to a reduction in OM, C and N concentrations. More C and N in the mineral soil likely implies improved growth conditions for roots and decomposing organisms, while physical rooting improves pore structure, water infiltration rate, and water holding capacity. Risch et al. (2010), working within some of the same stands our study was

conducted in, reported higher fine root and microbial biomass for the top 45 cm of the mineral soil after boar rooting. Besides the pure mixing effects they explained their findings by decreases in soil aggregate size, bulk density and potential changes in the composition of root herbivores, bacterial feeders, other soil animals and fungi as a result of the consumption of soil inhabiting vertebrates and invertebrates by the boars.

Similar to our results, higher OM (Lacki and Lancia, 1983) and N (Singer et al., 1984) concentrations, as well as lower bulk densities (Singer et al. 1984) were also reported for O, A and B horizons (to a depth of 25 cm) from rooted compared to non-rooted plots located in Great Smoky Mountains National Park (USA). The authors explained their findings with enhanced litter decomposition rates as a result of reduced particle size of litter residues due to rooting. Smaller organic particles increase the total surface area of OM in the soil, which in turn facilitates the absorption of soil moisture and increases the contact area for soil organisms (Singer et al., 1984). Similar to wild boars, digging by grizzly bears (Tardiff and Stanford, 1998) and pink-footed geese (van der Wal et al., 2007) in subalpine meadows and tundra ecosystems, respectively, was reported to positively affect ammonium-N and nitrate-N availability. Other authors working on wild boar rooting did not report any differences in OM, C and N concentrations (Groot Bruinderink and Hazebroek, 1996; Moody and Jones, 2000; Mohr et al., 2005) or bulk density (Groot Bruinderink and Hazebroek, 1996) between rooted and non-rooted plots. Mohr and Topp (2001) even found decreases in soil organic C and total N after boars rooted the soils of very steep (inclination between 24 and 34°) oak stands in Germany. They explained their findings with soil erosion leading to a loss of the freshly rooted material in the steep terrain, resulting in lower C and N values. Further reasons for the highly variable and contrasting results of the effect of rooting could be related to differences in ecosystem properties (e.g., vegetation cover, soil infiltration rates, soil physical, chemical and biological properties), boar densities, rooting intensities or climatic conditions. Also differences in soil sampling (e.g., sampling depths, sampling standard depths vs. sampling horizons) between the studies, or the time of sampling in relation to the initial rooting event could account for the different results found.

Rooting effects on soil OM, C, and N pools

In this study, rooting only affected OM, C or N pools in the litter layer, but not those in the mineral soil. The decreases in litter layer pools appear to be due to the incorporation of the material into lower soil horizons as a result of rooting. The reason for not detecting any differences in mineral soil pools despite the fact that we found significantly higher OM, C and

N concentrations, in turn, is related to the changes in bulk density we observed. When rooting, boars not only incorporate litter, but also mix air into the mineral soil layer. Consequently, when sampling to fixed soil depths, the decreases in bulk density and increases in OM, C and N concentrations counteract one another resulting in no differences in pools. Risch et al. (2010) found higher soil respiration rates on rooted compared to non-rooted plots in some of our stands indicating that C is lost from the system. Accelerated decomposition rates theoretically could be the reason for these losses. However, Risch et al. (2010) reported higher root and microbial biomass on rooted (compared to non-rooted) plots, indicating that the litter mixed into the mineral soil became mineralized and incorporated into biomass. This freshly produced biomass likely is more active and therefore produces more CO₂, without leading to losses in mineral soil C and N pools. When adding total pools across the entire soil profile studied in our project, short-term effects of wild boar rooting (measured approximately 3 months after the initial rooting event) resulted in negligible and non-significant differences in OM (-4.5%), C (+2.9%), and N (+3.8%) pools between the rooted and non-rooted plots. These findings indicate that wild boars probably do not alter the short-term C sink-source dynamics of forest ecosystems they inhabit. However, the effects of increased OM, C and N concentrations and decreased soil bulk density (e.g. improved growth conditions for trees, other vegetation and microbes) may have long-term consequences for the accumulation and release of C within the stands under study.

Unfortunately, there is scant literature that demonstrates calculated differences in OM, C and N pools after boar rooting and therefore we cannot directly compare our results. However, rooting by boars within forest stands may be broadly comparable to site preparation after harvesting or to plowing an agricultural field. For example, Johnson (1992) reviewed data from a variety of studies to examine, among other factors, how site preparation affects soil C pools. This review found a net loss of soil C after harvesting and/or site preparation (sampling intervals ranged from 1 month to 83 years after treatment), with the magnitude of loss depending on disturbance severity. However, most of the studies included in Johnson's review looked at clear-cut sites, which likely respond somewhat differently than our stands where forest cover was maintained. Similarly, Angers and Eriksen-Hamel (2008) reviewed literature in which soil organic C concentrations and pools were measured under no-till and full-inversion tillage in agricultural fields. They showed significantly greater soil organic C concentrations and pools under full-inversion tillage compared to no-till for the soil layer at mean plowing depth (23 cm), indicating that tillage has similar effects on agricultural soils as rooting by wild boars does within the forest.

Extrapolation of the rooting effect on C and N dynamics in Swiss forests

Roughly 410 km² or 11% (Risch et al., 2010) of Switzerland's 3711 km² hardwood/mixed hardwood forests (Brassel and Brändli, 1999) have similar boar densities as our stands. If we assume that on average 40.5% of the soil surface in these stands is affected by boar rooting (Risch et al., 2010), a total of 166 km² of hardwood/mixed hardwood stands would be similarly affected by boars as our stands. Given the changes in soil properties we found in the mineral soils of our study sites, boars potentially impact forest ecosystems on a much larger scale than just the stand level. They could, for example, improve growth rates of trees as shown by Lacki and Lancia (1986), who found that rooting positively affected shoot elongation of individual trees in American beech stands (Great Smoky Mountains National Park, USA) as a result of accelerated nutrient cycling and soil aeration. In contrast, the decrease in litter due to rooting may result in higher levels of erosion and the loss of valuable topsoil from the site (Mohr and Topp, 2001). This alteration in soil depth could ultimately result in decreased vegetative growth. Clearly, more detailed measurements on the impact of wild boars on ecosystem processes is needed to fully explain the long-term impacts of increased rooting in Swiss forests. This is particularly so since the impact of boars within Switzerland may increase in the near future as their population increases and their range expands within the country.

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Paper II

Effects of wild boar (*Sus scrofa* L.) rooting on the bacterial community structure in mixed-hardwood forest soils in Switzerland

Submitted as:

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Abstract

Soil disturbances are known to influence the soil microbial community structure and therefore have the potential to affect forest ecosystem functioning and productivity. Whereas most studies have focused on how disturbances originating from forest management practices alter these communities, almost nothing is known about the effects of biotic natural disturbances, especially the ones caused by large animals. Our goal was to determine how European wild boars (*Sus scrofa* L.) affect soil bacterial community structure and microbial biomass carbon by rooting (grubbing) in the soil when searching for food. We sampled microbial biomass carbon immediately and 24 months after the rooting event and determined bacterial community structure immediately, 11, 14 and 18 months after rooting on paired rooted and non-rooted study sites in four hardwood forest stands. In addition, we measured plant available total nitrogen, soil moisture and soil temperatures for each sampling interval. Wild boar rooting had no significant effect on soil bacterial community structure, diversity, richness and evenness or on microbial biomass carbon. However, we found that the bacterial community structure varied significantly with the sampling date. Correlations between bacterial community structure and different environmental parameters, namely plant available total nitrogen, soil moisture, and soil temperature were found to be responsible for these seasonal differences. Thus, seasonal changes in microbial community structure seem to override rooting effects of wild boars at our study sites.

Keywords

Bioturbation, rooting, soil nutrients, microorganisms, temperate forest

Introduction

Bacterial communities play an important role in forest soils as they affect ecosystem processes such as nutrient cycling, soil fertility and productivity, decomposition, soil carbon storage, or ground water quality (e.g. Nannipieri et al. 2003; Acea and Carballas 1996). No other natural community reacts as rapidly to fluctuations in habitat conditions as microorganisms do (Busse et al. 2006; Williamson and Wardle 2007). Their reactions to disturbance can range from resistance (composition stays the same, e.g., Balser and Firestone 2005), resilience (composition changes for a short period, but returns to original setting, e.g. Stark et al. 2007), functional redundancy (composition changes, but performs like original community, e.g., Wohl et al. 2004) to complete alteration (community and function change, e.g., Griffiths et al. 2000). Only the latter reaction will substantially affect ecosystem processes (Allison and Martiny 2008).

Research on disturbance effects of forest management practices such as tree harvesting (e.g., Sundman et al. 1978; Lundgren et al. 1982; Barbhuiya et al. 2004), site preparation (e.g., Staddon et al 1997; Lalor et al. 2007; Esquilin et al. 2008), prescribed burning (e.g., Donegan et al. 2001; Banning and Murphy 2008), or other anthropogenic impacts (alterations of soil chemical contents, heavy metal contamination; e.g., Bååth et al. 1995; Acea and Carballas 1996; Pennanen 2001; Compton et al. 2004; Frey et al. 2006) on soil microbial biomass carbon, activity and community structures (=composition and diversity) have revealed highly variable responses ranging from resistance to complete alteration in forest soils. The same holds true for large scale (landscape level) abiotic natural disturbances such as fire and uprooting of trees by wind throw; these disturbances can affect bacterial communities in forest soils directly (e.g. through soil heating; Pietikäinen et al. 2000), or indirectly through changes to the soil environment (e.g. Esquilin et al. 2007; Hamman et al. 2007).

The indirect effects of large-scale biotic natural disturbances -- in particular the ones caused by browsing or grazing animals -- on microbial biomass and/or activity have been frequently assessed (e.g. Pastor et al. 1988; Stark et al. 2000; Wardle et al. 2001). However, considerably fewer studies investigated how animals can directly alter these parameters through grubbing or trampling (e.g. Buckeridge and Jefferies 2007). Little is known about how animals affect the structure of microbial communities within forest soils: one study focused on the effects of

earthworms on soil microbial community structures under controlled conditions using soil microcosms (Saetre 1998) and a field study investigated the impact of insects and rodents on the structure of soil bacterial and fungal communities (Wardle et al. 2010). The potential impact of large animals on soil microbial community structures in forested areas has been completely neglected, which is surprising since large animals are ubiquitous in most forested ecosystems (Mohr et al. 2005).

For example, the omnivorous wild boar (*Sus scrofa* L.) – a species whose populations have grown substantially in many European countries during the past two decades (Boitani et al. 1995; Schley et al. 1998; Geisser and Reyer 2005; Hebeisen et al. 2008) – obtains a considerable proportion of its diet by rooting (grubbing) in the soil searching for plant seeds, roots/bulbs, and vertebrate and invertebrate animals (Howe and Bratton 1976; Wood and Roark 1980; Bratton et al. 1982; Baber and Coblentz 1987; Hone 1988). Thus, boars are a considerable agent of natural disturbance (bioturbation) in forest soils, since rooting involves breaking through the vegetation, typically affecting the top 15 cm to 70 cm of the litter layer and mineral soil (Imeson 1977; Genov 1981; Kotanen 1995) and disturbing up to 80 % of the forest soil surface (Genov 1981; Howe et al. 1981; Risch et al. 2010). Several authors have assessed how rooting affects forest soil physical, chemical and biological properties and have shown alterations of soil C and nutrient contents (e.g., Lacki and Lancia 1983; Moody and Jones 2000; Mohr et al. 2005; Risch et al. 2010), and decomposition and mineralization rates (e.g., Singer et al. 1984; Groot Bruinderink and Hazebroek 1996; Mohr and Topp 2001; Mohr et al. 2005). Rooting related changes in microbial biomass carbon and bacterial community structures could be largely responsible for these changes in ecosystem properties. Indeed, Risch et al. (2010) found higher microbial biomass carbon on rooted compared to non-rooted plots for the 0 to 45 cm mineral soil depths of mixed hardwood forests in Switzerland, while Mohr et al. (2005) reported lower microbial biomass carbon on rooted plots for the 0 – 5 cm soil depth of an oak forest in Germany. However, we are not aware of any studies that have investigated how rooting by wild boars affects the structure of soil bacterial communities.

The main objective of this study was to assess the structure of the bacterial community structure of the 0 to 15 cm mineral soil at different times of the year on rooted and non-rooted plots in the same mixed-hardwood forests where Risch et al. (2010) conducted their study. We also determined microbial biomass carbon for this soil depth to assess whether changes in community structure were paralleled by changes in microbial biomass. Finally, since environmental parameters play a dominant role regarding the structure of the general soil

bacterial community (Högberg et al. 2007), we were interested if the bacterial community structure was related to available mineral soil nitrogen, soil temperature, and soil moisture.

Materials and Methods

Study area and sampling design

The study was conducted in four mixed hardwood stands growing on sandstone parent material at elevations ranging from 550 to 670 m a.s.l. in northern Switzerland (Table 1). These stands were dominated by beech (*Fagus sylvatica* L.) but also contained other hardwoods [including oaks (*Quercus* ssp.) and hornbeam (*Carpinus betulus* L.)] and conifers [including Norway spruce (*Picea abies* L. Karst) and Scots pine (*Pinus sylvestris* L.)]. All sites were classified as typical *Galio odorati-Fagetum* following Keller et al. (1998) with litter layer depth ranging between 3.0 and 5.5 cm. We established a paired plot design consisting of a rooted (disturbed) and non-rooted (undisturbed) area in each of the four stands. The non-rooted plot was randomly selected in close proximity to the rooting incident, but located at least 15 m from the rooted plot. Both plots were 10 x 10 meters in size and were fenced with 1.3 m high knotted mesh (Ursus 130/11/15; mesh size at 0-50 cm height = 10 x 15 cm; mesh size at 50-110 cm = 15 x 15 cm; mesh size at 110-130 cm = 20 x 15 cm; Hortima AG, Hausen, Switzerland) tightened around 15 cm square wooden posts (1.6 m long) to prevent boars (and roe deer) from entering. Two 2.5 mm diameter wires were affixed at 1.4 and 1.5 m height to prevent animals from jumping over the 1.3 m mesh.

Table 1. Site and soil characteristics of the top 30 cm of the mineral soil for the 4 sites under study.

Site	Elevation	pH	Available water (g / 100 g soil)	Soil particle size distribution			Soil textural class
	(m)			Sand (%)	Silt (%)	Clay (%)	
1	550	3.2	10.7	72.4	17.7	9.9	Sandy loam
2	560	2.9	18.1	74.5	16.7	8.9	Sandy loam
3	660	2.8	20.2	66.8	24.3	8.9	Sandy loam
4	670	2.7	10.6	68.9	21.9	9.3	Sandy loam

* Soil pH was measured on a 2:1 water soil paste.

Since rooting generally is visible for roughly three years in the forest types we worked in, our non-rooted plots had not been disturbed for at least three years. To determine if boars specifically select foraging sites within a homogeneous stand or randomly root in the soil, we established a third randomly chosen, non-rooted and non-fenced 10 x 10 m plot at each study

site at the beginning of the study (May 2006). All these unfenced plots were rooted by the end of the study, implying that boars do not specifically select the sites where they are rooting. Thus, we conclude that our fenced, non-rooted plots were sufficient controls to be compared with the corresponding rooted plots. However, we cannot rule out that some of the differences in soil properties that we measured already existed prior to rooting. Unfortunately, such differences could not be accounted for as it is not possible to predict where the boars are going to root and therefore “pre-treatment” sampling was not possible.

Soil sampling

Soil sampling was conducted just after plot establishment in early June 2006, the following year in May, August, and December (2007), and two years after plot establishment in early June 2008. At every sampling event we randomly collected three mineral soil cores (5 cm diameter) on each rooted and non-rooted plot at least one meter from the fence to avoid edge effects. Samples were taken at total depth of 15 cm, after removing the litter layer. Soil sampling was performed at fixed depth with a soil corer (AMS, American Falls, ID, USA) since no clear soil horizon delineation was possible after rooting. The samples were pooled for each plot and sampling date and were immediately put on ice in the field. After transferring them to the laboratory they were sieved through a 2 mm sieve and kept in the cold room for immediate processing.

Bacterial community structure

DNA extraction and 16S rDNA PCR amplification

Bacterial community structure was determined for the samples collected in June 2006 and all samples collected in 2007. DNA was extracted from two replicates from each soil sample. 500 mg of fresh soil was frozen in 1.3 ml of DNA extraction buffer (2% CTAB; 20 mM EDTA pH 8; 2 M NaCl; 100 mM Tris THAM pH 8; 2% PVP-40) and 750 mg glass beads of 0.10 – 0.11mm diameter (Lazzaro et al. 2006). Soil DNA was prepared by a bead beating procedure (Frey et al. 2009). Briefly, frozen soil samples were subjected to three repeated extractions using a bead beater (FP 120; Savant Instruments, NY). Supernatants were purified with one chloroform-isoamyl alcohol (24/1) extraction. DNA was recovered after ethanol precipitation and re-suspended in TE buffer (pH 8). DNA concentrations of samples were adjusted to 5 ng μl^{-1} with TE buffer containing bovine serum albumin (BSA; final concentration 3 $\mu\text{g } \mu\text{l}^{-1}$) and heated for 2 min at 95 °C to bind PCR inhibiting substances such as humic acid. Bacterial 16S ribosomal RNA genes were amplified by PCR using

fluorescently labeled (6-FAM) forward primer 27f and unlabeled reverse primer 1378r (Heuer et al. 1997) in a total volume of 50 μ l reaction mixture containing 10-50 ng of template DNA, 1 x PCR-buffer (Qiagen, Hilden, Germany), 2 mM $MgCl_2$, 0.2 μ M of each primer, 0.4mM deoxynucleoside triphosphate (Promega), 0.6 mg ml^{-1} BSA (Fluka, Buchs, Switzerland), and 2 U HotStar *Taq* polymerase (Qiagen). PCR amplification was performed with the following cycling conditions: an initial activating step for HotStar *Taq* polymerase (15 min at 95 °C), followed by 35 cycles with denaturation for 45 s at 95 °C, annealing for 45 s at 48 °C, and extension for 2 min at 72 °C, with final extension for 5 min at 72 °C. The PCR amplification was then ended by an additional final step at 72 °C for 5 min. PCR success was confirmed by electrophoresis of aliquots of PCR mixtures (5 μ l) on a 1% agarose gel in 1% TAE buffer. In every case, inhibition of the PCR reaction by soil DNA was not observed.

Terminal restriction enzyme fragment length polymorphism analysis (T-RFLP)

Following confirmation of successful PCR reaction by agarose gel electrophoresis, the PCR products were purified with the Montage PCR purification cleanup kit (Millipore Corporation, Billerica, MA). Purified PCR products were digested with 2 U of restriction endonuclease *MspI* (Promega) and incubated overnight at 37°C. Aliquots (5 μ l) of digestion products were verified on a 2% agarose gel in 1% TAE buffer. Prior to the T-RFLP analysis, digests were desalted with a Montage SEQ96 sequencing reaction cleanup kit (Millipore Corporation, Billerica, MA) according to the manufacturer's instructions. T-RFLP analyses were performed according to Frey et al. (2006). Two μ l of digested PCR products were analyzed along with 0.2 μ l of the internal size standard ROX500 (Applied Biosystems, Foster City, USA) and 12 μ l HiDi formamide (Applied Biosystems, Foster City, CA) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with 36-cm capillaries filled with POP-4 polymer. T-RFLP profiles were analyzed using Genotyper v3.7 NT (Applied Biosystems) with a signal threshold of 50 relative fluorescence units. Relative signal intensities within a profile were calculated by dividing signal intensities of each individual T-RF by the sum of all signal intensities in a profile (Blackwood et al. 2003). This normalization procedure allowed us to compare signal intensities among different samples.

Microbial biomass carbon

Soil microbial biomass carbon was determined for the samples collected in June 2006 and June 2008 using the substrate-induced method of Anderson and Domsch (1978). De-ionized water was added as needed to bring all samples to 60% water-filled pore space. The samples

where then incubated at room temperature for 10 days prior to analyses to allow spurious microbial activity associated with the sampling disturbance to subside. This incubation period was determined in a preliminary experiment as a minimum before microbial activity declined to a steady state (basal respiration). Microbial biomass carbon was determined using 25 g of mineral soil (dry-weight equivalent) and a glucose concentration of 5 g kg⁻¹ soil. CO₂ production was analyzed 1-2 h following the addition of glucose using a LI-COR 6200 gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Environmental parameters

In addition to the soil sampling and analysis outlined above, we also made in situ measurements of available mineral soil nitrogen, soil temperature, and soil moisture, which potentially influence bacterial community structure. To assess plant available total nitrogen (total nitrogen availability) in the mineral soil, Plant Root Simulator probes (PRSTM; Western Ag Innovations Inc., Saskatoon, SK, Canada) with ion-exchange resin membranes were incubated 6 weeks prior each soil sampling event. At four randomly selected locations per plot, one pair of PRSTM probes, consisting of an anion and a cation probe, respectively, were inserted vertically into the soil (always into the same location). Since it is important to avoid root competition (plant roots act as ion sinks, just as the PRSTM probes do) during long-duration burials, we cut a 30 cm deep x 2 mm wide slit around the probes (square of 30 x 30 cm) with a garden spade. Additionally, we continuously removed all plants growing in this square. In the lab, the PRSTM-probes were cleaned thoroughly using toothbrushes and deionized water and were stored in labeled freezer bags at 4°C in the cold room. At the end of the experiment all samples were sent to Western Ag Innovations Inc. laboratory for analysis. In addition, we measured soil temperature with a waterproof digital pocket thermometer (Barnstead International, Dubuque, IA, USA) and soil moisture with a Field Scout TDR 100 (time domain reflectometer; Spectrum Technologies, Plainfield, IL, USA) for the 0 to 10 cm mineral soil depth at five random points at each plot during each of the soil sampling events.

Statistical analyses

Relationships in bacterial community structure between rooted and non-rooted plots over time (seasonal effect) was determined by comparing T-RFLP profiles of each sample using multivariate statistical methods (Frey et al. 2006; Lazzaro et al. 2006). Since the initial detrended correspondence analysis (DCA) indicated that the data exhibited a linear, rather than a unimodal, response, we used principal component analysis (PCA) with the aim of

identifying samples that generate similar patterns (Leps and Smilauer 2003). To investigate whether bacterial community structure differed between the paired rooted and non-rooted plots over time, we used a linear mixed model procedure to avoid potential pseudoreplication of the data. Corresponding sample scores (intercepts of PCA axis 1 and 2) were a function of the two fixed effects, treatment (rooted and non-rooted) and time (seasonal effect), and the random effect site. We also adjusted these models for temporal autocorrelation where necessary. Further, total nitrogen availability, soil temperature, and soil moisture were added post-hoc to the ordination (PCA) by projection as supplementary environmental variables, resulting in a so-called sample-environment biplot. Biplot scores of environmental variables (which are precisely equal to the inter-set correlations in indirect ordination methods) and correlation coefficients between principal components and environmental variables were used to interpret the ordination (PCA). All the multivariate analyses were performed on the T-RFLP profiles according to ter Braak and Smilauer (2002) using CANOCO software for Windows 4.5 (Microcomputer Power, Ithaca, NY). For these analyses mentioned above we only used the 2007 data. The same linear mixed model approach as described above was used to test for treatment (rooted and non-rooted) and season (time) effects on richness, diversity and evenness of T-RFs. Richness refers to the number of distinct T-RFs detected in a given sample. Diversity was calculated using the Shannon index as follows:

$$\text{Shannon Diversity} = - \sum P_i \log P_i,$$

where $P_i = n_i/N$, n_i is the height of peak, and N is the sum of all peak heights in the curve. To measure how evenly the different T-RFs were distributed in a given soil sample, the evenness was calculated as follows:

$$\text{Evenness} = \text{Shannon Diversity} / \ln(\text{Richness}).$$

Differences in microbial biomass carbon between the rooted and non-rooted plots were tested using Wilcoxon signed-rank tests for paired samples. Alpha-level was 0.05 for all analyses.

Results

Rooting effects on bacterial community structure

The first two PCA axes (principal components) explained 53.4% of the variability in the bacterial community structure (Figure 1). We found no significant difference in bacterial community structure between rooted and non-rooted plots overall (axis 1: $t = -0.17$, $p = 0.87$; axis 2: $t = -1.52$, $p = 0.15$) and neither for any of the sampling occasions for any of the three diversity indices calculated (Figure 2). The PCA of T-RFLP profiling data revealed that the samples were separated according to their sampling date (seasonal effect) on axis 1 and that

those differences were highly significant ($t = 12.08$; $p < 0.001$). No significant differences were, in contrast, found on sample scores of axis 2 ($t = 1.14$; $p = 0.27$). Our data also showed that T-RFLP richness ($t = -3.24$, $p < 0.01$) and evenness ($t = 4.05$, $p < 0.001$) significantly changed over time; while no significant temporal differences were found for the T-RFLP Shannon diversity ($t = -0.51$, $p = 0.61$).

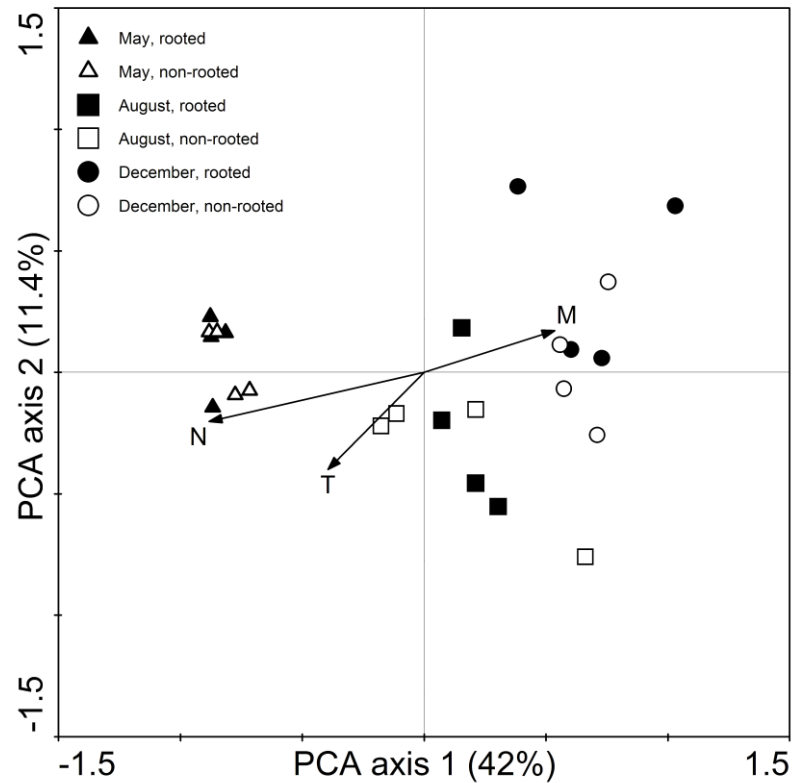


Figure 1: Principal component analysis of T-RFLP profiles of eight paired plots (rooted versus non-rooted) at 4 sites for three sampling dates (May, August and December). Post-hoc added supplementary environmental variables (M = soil moisture; N = total nitrogen availability; T = soil temperature) are indicated as arrows.

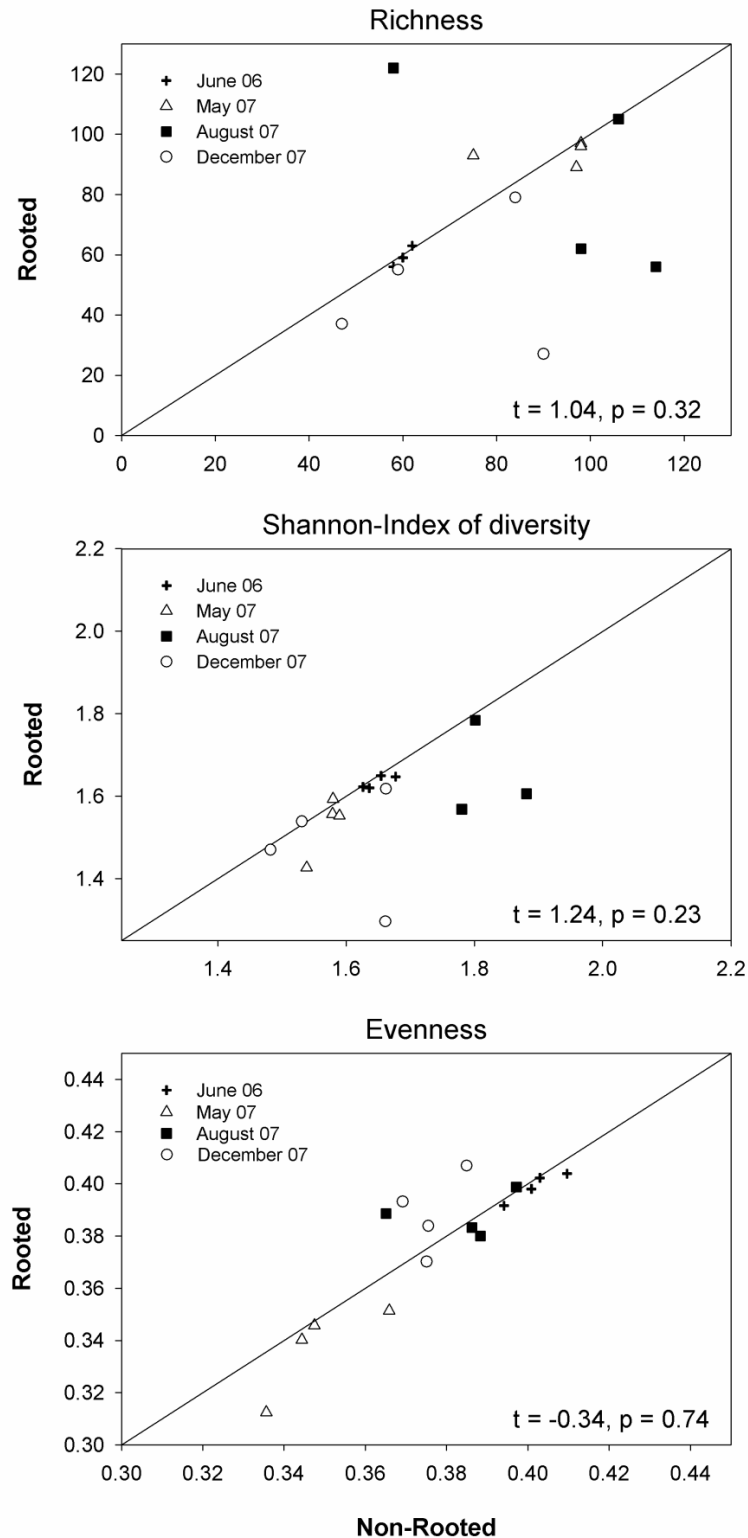


Figure 2: T-RFLP richness, Shannon-Index of diversity, and evenness of rooted and non-rooted plots measured in June 2006, and May, August, and December 2007 ($n = 4$ for each time). $t = t$ value, $p = p$ value of the treatment effect (linear mixed model procedure).

Rooting effects on soil microbial biomass carbon

Microbial biomass carbon in the top 15 cm of the mineral soil averaged $443.8 \pm 18.2 \text{ mg kg}^{-1}$ soil (dry weight) for rooted compared to $359.3 \pm 42.3 \text{ mg kg}^{-1}$ soil on the non-rooted plots in June 2006. These small differences were not statistically significant ($df = 3$, $t = 1.66$, $p = 0.20$; Figure 3). The same pattern was found for the second sampling date ($df = 3$, $t = 0.74$, $p = 0.52$; Figure 3): rooted microbial biomass carbon averaged $602.7 \pm 101.6 \text{ mg kg}^{-1}$ soil, the one on the non-rooted plots $511.3 \pm 49.8 \text{ mg kg}^{-1}$ soil.

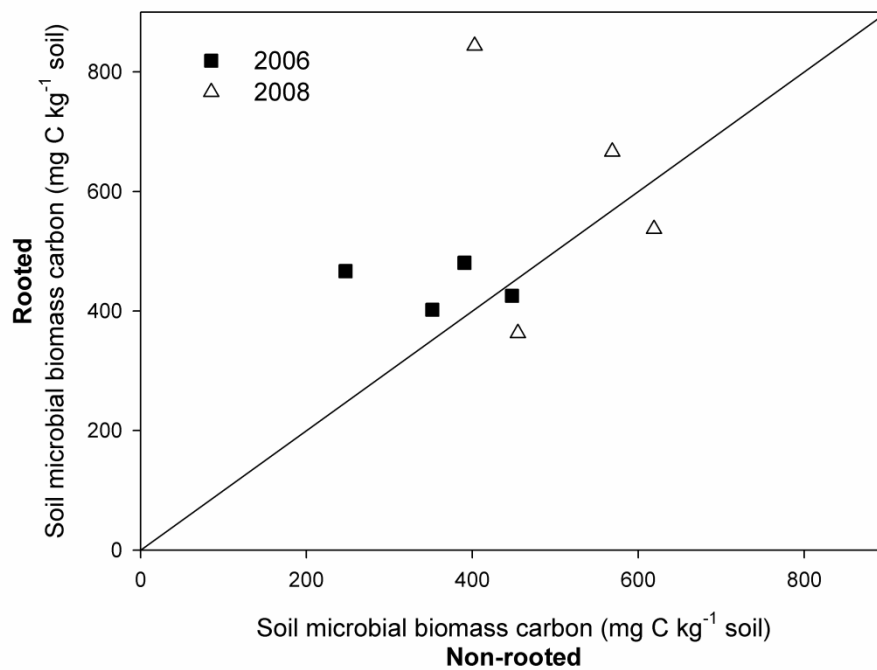


Figure 3: Microbial biomass carbon (mg C kg^{-1} soil) for the top 15 cm of the mineral soil on rooted and non-rooted plots measured in June 2006 ($n = 4$) and June 2008 ($n = 4$).

Correlations between environmental parameters and bacterial community structure

Post-hoc added supplementary environmental variables (resulting in a sample-environment biplot, Figure 1) explained up to 33.5% of the total variability in the bacterial community structure that we detected. The first principal component was significantly negatively correlated with total nitrogen availability ($r = -0.6846$, $p < 0.01$) and significantly positively correlated with soil moisture ($r = 0.4158$, $p = 0.04$), whereas no correlation was found with soil temperature ($r = -0.3047$, $p = 0.15$). Bacterial community structures in May, August, and December were significantly separated from each other due to differences in soil moisture, and total nitrogen availability. The second principal component was significantly (negatively)

correlated with soil temperature ($r = -0.5947$, $p < 0.01$), no correlation was found for soil moisture ($r = 0.2559$, $p = 0.23$) and total nitrogen availability ($r = -0.3012$, $p = 0.15$).

Discussion

Rooting effects on soil bacterial community structure

We could not find any statistically significant differences in mineral soil bacterial community structure or in the bacterial diversity indices (richness, Shannon Diversity, and evenness) between rooted and non-rooted plots. Consequently, our findings suggest that the bacterial community structure responded with resistance (structure stays the same) to rooting by wild boars. The idea that microorganisms are resistant, resilient, and functionally redundant is pervasive in ecology (Allison and Martiny 2008), with certain microbial groups showing high physiological tolerance to changing environmental conditions. A potential reason for the lack of response could be related to the fact that rooting did not alter soil pH in our study (unpublished data). Soil pH is known to play a dominant role in regulating the structure of the soil microbial community. Högberg et al. (2007), for example, found that soil pH (together with soil C-to-N ratio) influenced soil microbial community composition, resulting in increased bacterial phospholipid fatty acids with increasing pH.

Unfortunately, we were not able to find any literature that assessed how disturbance by large animals affects soil microbial community structures in forest soils, for comparison with our findings. The only two studies that included the effects of animals on microbial community structures investigated how these communities respond to the effects of earthworms and insects, in combination with rodents. Saetre (1998) found that earthworm additions into his microcosm were responsible for the observed changes in microbial community structures, while Wardle et al. (2010) reported no differences in microbial community diversity regardless of the addition of substrates (nitrogen and artificial honeydew) or presence – absence of rodents. We hesitate to compare these results with ours, because soil mixing activities by earthworms or burrowing rodents probably influence soil ecosystem properties differently than the unobtrusive rooting by wild boars does.

Forest management practices (site preparation) - especially soil scarification and contour ripping that mix the surface litter layer with the mineral soil – could, however, have a similar effect on soil microbial community structures as rooting by wild boars. Ohtonen et al. (1992) found no significant effect of soil scarification on bacterial community structures (measured as relative volume of bacteria to fungi) 5 yr after initial site preparation, while Staddon et al. (1997) reported significantly reduced functional diversity of soil microbial communities 4

years after soil scarification on different clear-cut sites in central Ontario, Canada. Similarly, Esquelin et al. (2008) demonstrated long-term (> 20 yr) impacts of forest soil scarification on microbial community structures of a ponderosa pine forest soil in the central Rocky Mountains. They explained their findings by lower amounts of total C and OM that lead to alterations of microbial communities towards a domination of Gram-positive bacteria in scarified soils. Three other authors investigated changes in microbial community structure in a forest rehabilitation study of a mine located south of Perth, Australia: Lalor et al. (2007) and Cookson et al. (2008) found significant effects of contour ripping on the microbial community level physiological profiles (CLPP) in ripped soils of the 3-, but not the 16-year old plots. They suggested that the open canopy of the 3-year-old rehabilitation (compared to the closed canopy of the 16-year-old rehabilitation) has led to greater fluctuations in soil water content and temperature, which may select for organisms capable of withstanding such conditions and thus altering microbial community structures. Banning and Murphy (2008) looked at DNA fingerprints to assess the effect of contour ripping on the microbial community structure at the same site, and also detected significant differences in bacterial community structure between micro-topographical positions (mounds and furrows) introduced by contour ripping.

Site preparation has often, but not always, been found to alter soil microbial community structures. This stands in clear contrast with our study, where soil disturbance by boars did not result in any statistically significant differences in the structure of the microbial community samples. For us, this is surprising, given the massive rooting of soils we have seen in the field, which results in a degree of disruption that is comparable to site preparation. One possible explanation for the different results could be that all the site preparation studies looked at clear-cut sites or forest rehabilitations, which likely respond somewhat differently than our stands where forest cover was maintained. An intact forest ecosystem may be able to buffer even severe soil disturbance (see Lalor et al. 2007, Cookson et al. 2008) whereas clear-cut sites or forest rehabilitations may be unable to do so.

Rooting effects on soil microbial biomass carbon

Our study did not reveal any rooting effect on soil microbial biomass for the 0 - 15 cm mineral soil depth, yet a tendency towards higher microbial biomass carbon on rooted plots was visible. Indeed, when extending the sampling to deeper soil depths, Risch et al. (2010) - working in the same stands - found higher microbial biomass carbon in the top 45 cm of the mineral soil on rooted compared to non-rooted plots. These findings suggest that rooting by wild boars positively influences the incorporation of organic matter from the litter layer into

the mineral soil, which increases resource availability (OM, C, and N content; Wirthner et al. submitted) and therefore favors the growth of the microbial communities. However, investigations conducted in steep oak forests in Germany revealed opposite findings compared to our findings and the results of Risch et al. (2010), with lower values for microbial biomass carbon on rooted compared to non-rooted loamy soils (0 - 5cm; Mohr and Topp 2001; Mohr et al. 2005). There are several possible reasons for these results. First, in contrast to the mixed hardwood stands we worked in, they found generally lower contents of organic C and total N at the rooted plots compared to the control plots, which may explain the lower microbial biomass they found at their rooted plots. Second, rooting could have led to accelerated nutrient leaching and consequently loss of resources for the microbes in the steep terrain they were working in. Similarly to the studies on wild boar rooting conducted in Germany, also grubbing by geese resulted in a decrease of soil microbial biomass in an Arctic salt marsh (Buckeridge and Jefferies 2007). The authors explained their findings by the loss of vegetation through rooting leading to a reduction of plant inputs (carbon) into the soil, which in turn reduced microbial growth.

In summary, the response of microbial biomass to biotic disturbances seems to be unpredictable and reflects the complexity of how soil disturbances affect the system, an ongoing challenge for ecologists (Wardle and Giller 1996). The reasons for this unpredictability likely stem from the diversity in site conditions, both abiotic (climate, soil development, disturbance intensity, soil physical and chemical properties) and biotic (forest type, growth rates, vegetation recovery, community structure), under which the studies were conducted, the factors that – as Busse et al. (2006) concluded – are responsible for our imperfect understanding of disturbance effects.

Seasonal changes in the soil bacterial community structure

Despite the fact that rooting did not significantly alter the bacterial community structure, our data revealed that bacterial communities significantly differed between the May, August and December sampling that we conducted. Thus, seasonal differences seem to have a considerably stronger effect on communities than rooting or site-to site differences in chemical and physical soil properties. Time was also the strongest factor predicting differences in microbial community structures in the other two studies, summarized above, that investigated the effect of animals on microbial communities (Saetre 1998, Wardle et al. 2010). Similarly, Moore-Kucera and Dick (2008) also reported that seasonal changes in soil microbial community were more pronounced than the effects of clear-cutting in the Gifford

Pinchot National Forest, Washington State, USA. Given the negative impact clear-cutting had on plant community structure and C inputs in their study, they also were somewhat surprised that the seasonal effect was more pronounced than clear-cutting.

Conclusions

Wild boar rooting did not have a significant effect on microbial community structures in the forest stands under study, which was surprising because these animals significantly disturb forest soils. Comparing our findings with other studies turned out to be difficult, as almost no literature is available on how animals alter microbial community structures. Consequently, our paper reveals how little we know about how disturbance by large animals affects these ecosystem parameters. Since large animals inhabit forest ecosystems around the world, it is important that we better understand their effects on how forest ecosystems function.

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Paper III

**Do changes in soil properties after rooting by wild boars (*Sus scrofa* L.)
affect understory vegetation in Swiss hardwood forests?**

Submitted as:

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Abstract

Recovering from small fragmented populations, wild boars (*Sus scrofa*) have considerably increased their numbers and habitat range in many European countries during the past two decades. Although several studies have focused on the impact of wild boar rooting on selected vegetation properties, little is known about effects on entire forest ecosystems. The main goal of our study was to assess how rooting by boars alters soil and vegetation properties, and how these effects of rooting interact with one another. We measured soil chemical and biological properties (carbon and nitrogen concentrations, nitrogen availability, and microbial biomass carbon), as well as several vegetation characteristics (total plant cover, plant species diversity, number and height of saplings) on paired rooted and non-rooted plots in six hardwood forests in Switzerland.

We found that rooting by wild boars led to significant increases in mineral soil carbon and nitrogen concentrations and microbial biomass carbon, which could lead to improved growth conditions for plants. However, total plant cover and sapling counts were reduced on rooted plots, possibly due to mechanical disturbance, or due to reduced plant available nitrogen (measured as supply rate, in contrast to the observed increase in total stocks of mineral soil N). In view of these results, simple characterizations of wild boar rooting as beneficial or detrimental to forest ecosystems should be handled with care.

Keywords

Grubbing, plant properties, soil nutrients, organic matter, temperate forest

Introduction

At the beginning of the last century wild boars (*Sus scrofa* L.) were close to extinction throughout much of their natural range in Eurasia. Recovering from small, fragmented populations, wild boar have increased their numbers and their habitat range considerably in many European countries, including Switzerland, during the past two decades (Apollonio et al. 2010). Reasons for this widespread expansion include boars' opportunistic feeding behavior, high reproductive potential, and adaptability to a wide variety of habitats (*i.e.*, high ecological plasticity; Boitani et al. 1995; Taylor et al. 1998). Wild boars are omnivorous and obtain a considerable proportion of their diet by rooting (grubbing) in the soil searching for plant seeds, roots, bulbs and vertebrate and invertebrate animals (*e.g.* Howe and Bratton 1976; Wood and Roark 1980; Bratton et al. 1982; Baber and Coblenz 1987; Hone 1988). Rooting involves breaking through the surface layer of vegetation followed by excavation of the detected food item, thereby disturbing up to 80 % of the soil surface (Genov 1981; Howe et al. 1981; Risch et al. 2010). Excavation may be superficial, affecting the surface organic horizons only, but rooting typically affects the organic horizons and mineral soil combined, to depths of 15 cm or more (Genov 1981; Kotanen 1995).

In temperate forests, boar rooting can have a large impact on carbon (C) and nutrient cycling. The addition of energy rich organic material mixed into the mineral soil alters C and nutrient concentrations (Nannipieri et al. 2003), and can stimulate microbial growth and activity (Mallik and Hu 1997) and therefore can accelerate decomposition processes. Only a few researchers have investigated how rooting habits of wild boars affect forest soil C concentrations, nutrient cycling processes, or soil microbial properties (Lacki and Lancia 1986; Groot Bruinderink and Hazebroek 1996; Moody and Jones 2000; Mohr and Topp 2001; Mohr et al. 2005; Siemann et al. 2009; see Table 1). These changes in soil properties could indirectly impact understory vegetation and/or sapling growth, and wild boar rooting may also directly (and adversely) affect or damage the understory vegetation (through, *e.g.*, seed predation and mechanical damage). Several authors have studied how wild boar or feral hog populations alter understory plant cover (Bratton 1974, 1975; Singer et al. 1984; Peart and Patten 1992; Siemann et al. 2009; Cuevas et al. 2010), plant community structure (Bratton 1975; Aplet et al. 1991; Peart and Patten 1992, Siemann et al. 2009), and plant regeneration

and growth potential (Lacki and Lancia 1986; Ickes et al. 2001; Mitchell et al. 2007; Gomez and Hodar 2008; Sanguinetti and Kitzberger 2010; Siemann et al. 2009; see Table 1). In contrast, we only found three studies that investigated both above- and belowground parameters (Singer et al. 1984; Lacki and Lancia 1986; Groot Bruinderink and Hazebroek 1996, see Table 1). These authors assessed the effects of wild boars on soil nitrogen (N) and/or organic matter (OM) concentrations as well as plant cover, regeneration and growth potentials, but none of these studies included nutrient dynamics and soil microbial properties. The goal of our study was to provide a more comprehensive understanding of how boar rooting might affect the entire plant-soil system, by investigating rooting effects on soil chemical and biological properties (C and N concentrations, N availability, and microbial biomass carbon) as well as understory vegetation characteristics (total plant cover, plant species diversity, number and height of saplings) in mixed hardwood forests, the forest types most frequently used as boar habitat in Switzerland.

Table 1. Studies reporting wild boar rooting effects on above and belowground properties and processes (N conc. = soil nitrogen concentration; N flux = soil nitrogen availability; C conc. = soil carbon concentration; Micro. bm = soil microbial biomass carbon; Plant comm. = plant community structure; Forest regen. = forest regeneration potential; + = positive effect; - = negative effect; 0 = no effect found; blank = property not measured)

Forest type	Wild boars	N conc.	N flux	C conc.	Micro. bm	Total plant cover	Plant comm.	Plant growth	Forest regen.	Source	Location
Mixed hardwood forest	native	+	-	+	+	-	0	0	-	This study	Switzerland
Gray beech forest	non-native					-				Bratton (1974)	Tennessee, North Carolina
Gray beech forest	non-native					-	- / 0			Bratton (1975)	Tennessee, North Carolina
Savannah, grassland, pine/shrub thicket	non-native						0		0	Baron (1982)	Mississippi
Rain forest	non-native					0			-	Ralph and Maxwell (1984)	Hawaii
Hardwood forest	non-native	+ / -				-				Singer et al. (1984)	Tennessee, North Carolina
Hardwood forest	non-native			+	(OM)			+		Lacki & Lancia (1986)	Tennessee, North Carolina
Hardwood (rain) forest	non-native						+ / - / 0			Aplet et al. (1991)	Hawaii
Hardwood forest	non-native					-	*		-	Peart & Patten (1992)	California
Grassland & conifer forest	native						+			Welander (1995)	Sweden
Mixed hardwood and conifer forest	native	0		0	(OM)			0	- / 0	Groot Bruin. & Hazebroek (1996)	Netherlands
Hardwood forest	non-native	0 (nitrate)		0						Moody & Jones (2000)	California
Hardwood (rain) forest	native						+	- / 0	-	Ickes et al. (2001)	Malaysia
Hardwood forest	native	-		-	-					Mohr & Topp (2001)	Germany
Oak woodland	non-native								-	Sweitzer and Vuren (2002)	California
Hardwood forest	native	0		0	- / 0					Mohr et al. (2005)	Germany
Hardwood (rain) forest	non-native								-	Mitchell et al. (2007)	Australia
Mixed woodland	native								-	Gomez & Hodar (2008)	Spain
Mixed forest	non-native					-	+	-	0	Siemann et al. (2009)	Texas
Mast conifer forest	non-native								-	Sanguinetti & Kitzberger (2009)	Argentina
Hardwood woodland, shrubs, dunes	non-native					+ / - / 0				Cuevas et al. (2010)	Argentina
Mixed hardwood forest	native				+					Risch et al. (2010)	Switzerland

Materials and Methods

Study area and sampling design

The study was conducted in six beech (*Fagus sylvatica* L.) dominated forests that also contained other hardwoods such as oaks (*Quercus* spp.) and hornbeam (*Carpinus betulus* L.) and conifers [Norway spruce (*Picea abies* L. Karst); Scots pine (*Pinus sylvestris* L.)], located in northern Switzerland. All six sites were classified as typical *Galio oderati-Fagetum* following Keller et al. (1998) and were located within 50 km of each other, with the closest distance between two sites exceeding 1 km. Elevations ranged from 550 to 670 m a.s.l. All sites were located on sandy-loam textured soils with soil pH ranging between 2.7 and 3.6 (Table 2). Each forest site was inhabited by wild boars throughout the year and rooting was widespread. We established a paired-plot design consisting of a rooted (disturbed) and undisturbed plot in each of the six forests in early spring of 2006. Both plots were 10 x 10 meters in size and were fenced with 1.3 m high knotted mesh (Ursus 130/11/15; mesh size at 0-50 cm height = 10 x 15 cm; mesh size at 50-110 cm = 15 x 15 cm; mesh size at 110-130 cm = 20 x 15 cm; Hortima AG, Hausen, Switzerland) tightened around 15 cm square wooden posts (1.6 m long) to prevent boars (and roe deer) from entering. Two 2.5 mm diameter wires were affixed at 1.4 and 1.5 m height to prevent other animals from jumping over the 1.3 m mesh.

Table 2. Site and soil characteristics of the top 15cm of the mineral soil for the six sites under study.

Site	Elevation (m)	pH	Available water (g / 100 g soil)	Rock content (%)	Soil particle size distribution			Soil texture class
					Sand (%)	Silt (%)	Clay (%)	
1	550	3.12	20.41	41.90	73.2	18.0	8.8	Sandy loam
2	550	3.56	12.72	41.79	74.4	16.1	9.5	Sandy loam
3	560	2.81	12.60	33.46	75.8	17.1	7.1	Sandy loam
4	610	2.68	21.36	41.00	72.5	21.0	6.6	Sandy loam
5	650	2.69	21.09	17.58	67.2	26.0	6.8	Sandy loam
6	670	2.59	10.93	55.65	69.2	22.8	8.1	Sandy loam

*Soil pH was measured on a 2:1 water soil paste.

Since rooting is generally visible for approximately three years in these forest types, our undisturbed plots had not been rooted for three years. To determine if boars specifically select foraging sites within a homogeneous stand or randomly grub in the soil, we established a third randomly chosen, non-rooted and non-fenced 10 x 10 m plot at each study site at the beginning of the study (May 2006). These random plots were all rooted by boars by the end of

the study and, therefore, we assume that boars do not key in on specific habitat characteristics when they forage. Thus, our randomly chosen non-rooted fenced plots were sufficient to be compared with the respective rooted plots. However, we cannot rule out that some of the differences in ecosystem properties that we measured already existed prior to rooting. Unfortunately, such differences could not be accounted for, because it is not possible to predict where the boars are going to root and therefore “pre-treatment” sampling was not possible (see also Risch et al. 2010).

Soil sampling and analyses

Soil sampling was conducted just after plot establishment in early June 2006 (time = 0 months), and again two years (time = 24 months) after plot establishment. During each sampling campaign, we randomly collected three mineral soil cores (5 cm diameter) from each rooted and non-rooted plot, at least one meter from the fence to avoid edge effects. Samples were taken across a total depth of 45 cm, after removing the organic horizons. Soil sampling was conducted at fixed depths with a soil corer (AMS, American Falls, ID, USA) since no clear soil horizon delineation was possible after rooting. Sub-samples were oven dried at 65 °C (for 48 h), passed through a 2 mm sieve, and finely ground. Total C and N concentrations were analyzed on a LECO induction furnace at 950 °C (LECO Corporation, St. Joseph, Michigan, USA). Values obtained from the three samples at each depth were averaged for each plot.

Microbial biomass was determined at 0 and 24 months using the substrate-induced method of Anderson and Domsch (1978). Fifty gram sub-samples from all samples (pooled for each plot and sampling date) were immediately put on ice when collected, sieved through a 2 mm sieve and stored in the cold room at 4 °C until further processing. De-ionized water was added as needed to bring all samples to 60% water-filled pore space. The samples were then incubated at room temperature for 10 days prior to analysis, to allow spurious microbial activity associated with the sampling disturbance to subside (to measure only basal respiration). Microbial biomass carbon was determined using 25 g of mineral soil (dry-weight equivalent) and a glucose concentration of 5 g kg⁻¹ soil. CO₂ production was analyzed 1-2 h following the addition of glucose using a LI-COR 6200 gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Nitrogen availability

To assess plant available total N (N supply rate as the sum of ammonium and nitrate) in the mineral soil, we incubated Plant Root Simulator probes (PRSTM; Western Ag Innovations Inc., Saskatoon, SK, Canada) with ion-exchange resin membranes. At four randomly selected locations per plot, one pair of PRSTM probes, consisting of one anion and one cation probe, were inserted vertically into the soil. To ensure that membranes do not become saturated with ions absorbed from the soil, the probes were replaced with new probes (inserted into the same locations) every six weeks (time = 0 until time = 24 months; number of measurements = 15). Since it is important to avoid root competition (plant roots act as ion sinks, similarly to the PRSTM probes) during long-duration burials, we cut a 30 cm deep x 2 mm wide slit with a garden spade around the probes (square of 30 x 30 cm). Additionally, we continuously removed all plants growing within this square. In the lab, the PRSTM-probes were cleaned thoroughly using toothbrushes and deionized water, and were stored in labeled freezer bags at 4°C in the cold room. At the end of the experiment all samples were sent to Western Ag Innovations Inc. laboratory for analysis.

Vegetation sampling

All understory plant species were identified and the cover fraction of each species was quantified by visual estimation on four randomly selected 1 x 1 m sub-plots within each rooted and non-rooted plot, for three points in time: the initial survey after plot establishment at the beginning of the study (time = 0), and 12 months (time = 12) and 48 months (time = 48) after later. Within each treatment, values of the four subplots were averaged. Plant species diversity was calculated as the Shannon Index of diversity. Sapling abundance was determined by counting all saplings (less than 100 cm tall) and current-year seedlings. We also estimated total plant cover and measured the individual height of each sapling growing in each plot to the nearest centimeter. Height measurements of saplings at time = 12 and time = 48 were restricted to individuals taller than 10 cm to exclude seedlings grown after the initial survey. Saplings were not individually tagged.

Statistical analyses

A linear mixed model procedure approach was used to test for treatment (rooted and non-rooted) and season (time) effects on mineral soil C and N concentrations, microbial biomass, N availability, total plant cover, plant species diversity, number of saplings, as well as height of saplings. These parameters were a function of the two fixed effects, treatment (rooted and

non-rooted) and time, and the random effect site. We also adjusted this model for temporal autocorrelation. Because the vegetation data were strongly skewed and the sample sizes were small, the assumptions underlying traditional parametric statistics were not met. Therefore, more robust non-parametric analyses were used instead, i.e. the same linear mixed model approach as described above, but ranked data (fractional rankings) were used instead of the original parametric values. All statistical analyses were performed with the R statistical package: Version 2.4.1, R, Development Core Team, 2006).

Results

Rooting, C and N concentration and fluxes, and soil microbial properties

Mineral soil C and N concentration and microbial biomass C (0 to 45 cm depth) were significantly higher on the rooted compared to the non-rooted plots. Time effects could only be found for the microbial biomass C, with significantly higher values measured two years after plot establishment ($t = 24$) compared to values measured immediately after plot establishment ($t = 0$, Figure 1-3). Nitrogen availability was significantly lower on the rooted plots (mean \pm standard error: $115.87 \pm 23.74 \mu\text{g } 10 \text{ cm}^{-2} \text{ 6 weeks}^{-1}$) compared to the non-rooted plots ($160.06 \pm 29.37 \mu\text{g } 10 \text{ cm}^{-2} \text{ 6 weeks}^{-1}$; no. of measurements in each plot = 15) and varied significantly between different sampling dates (Figure 4).

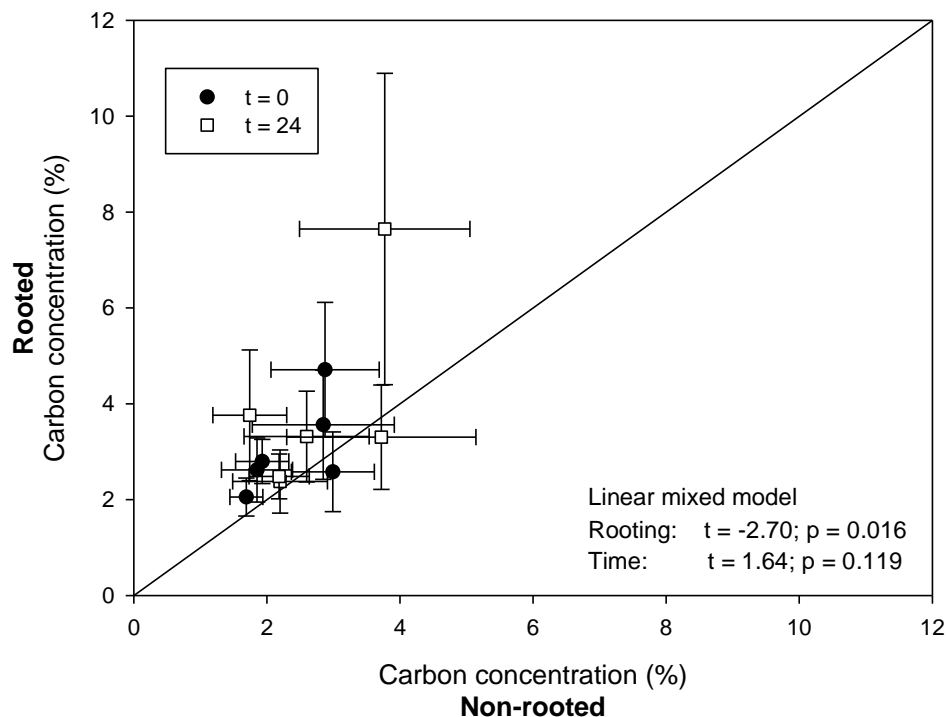


Figure 1: Carbon concentration for the top 45 cm of the mineral soil on rooted and non-rooted plots measured at time = 0 and time = 24 months ($n = 6$). Data represents averages \pm standard errors of all sites.

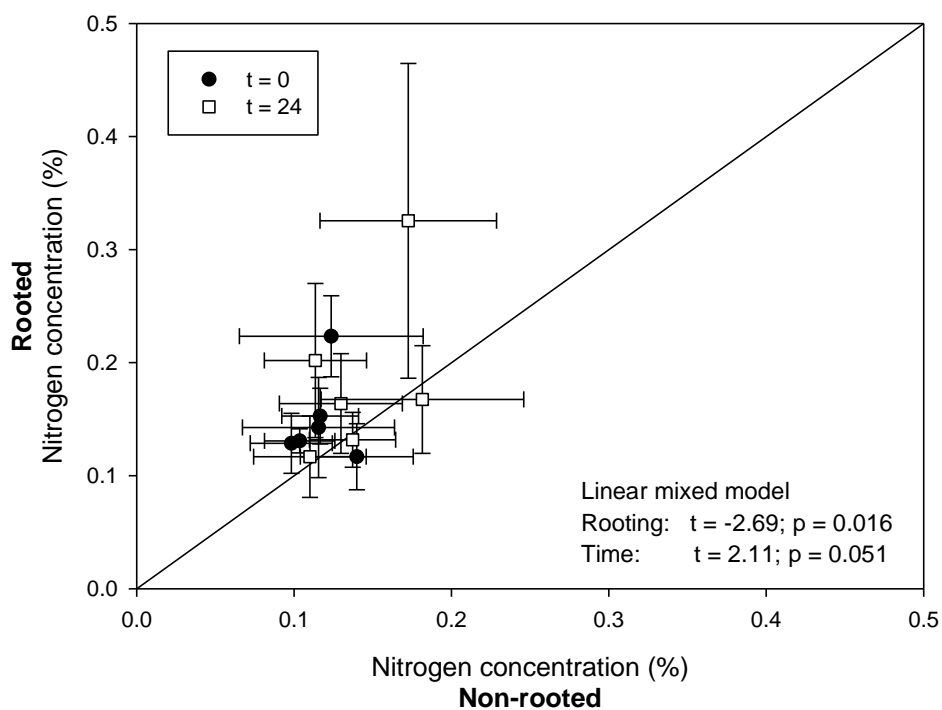


Figure 2: Nitrogen concentration for the top 45 cm of the mineral soil on rooted and non-rooted plots measured at time = 0 and time = 24 months ($n = 6$). Data represents averages \pm standard errors of all sites.

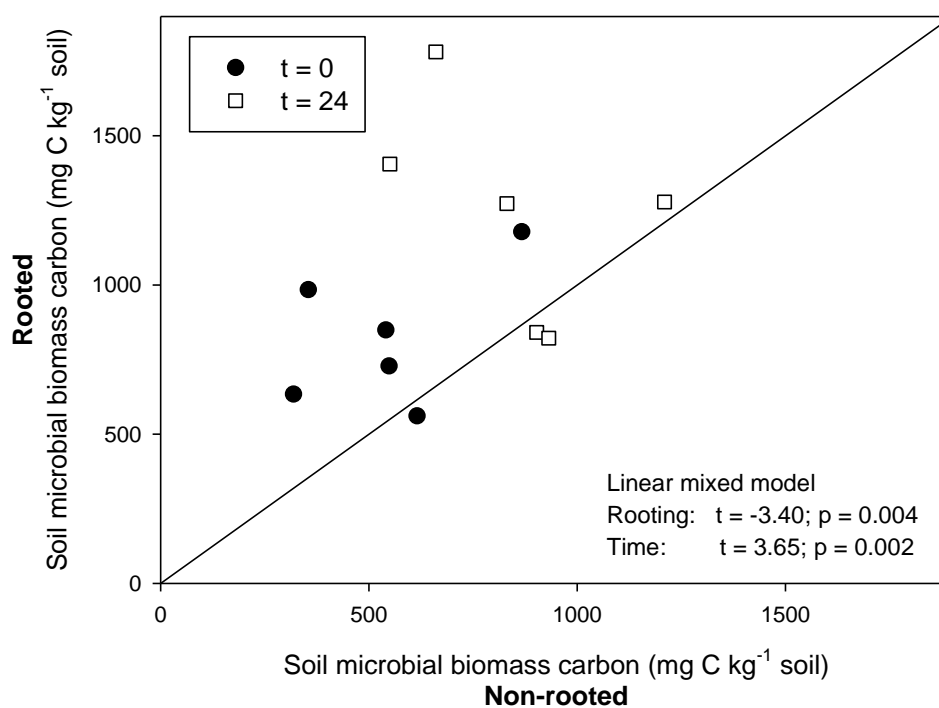


Figure 3: Microbial biomass carbon for the top 45 cm of the mineral soil on rooted and non-rooted plots measured at time = 0 and time = 24 months ($n = 6$).

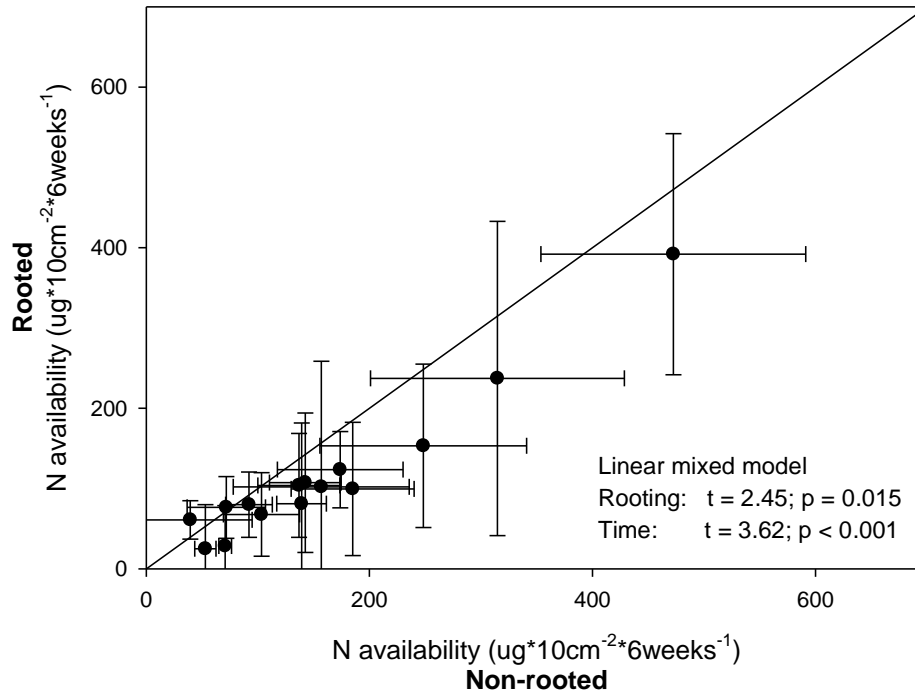


Figure 4: Plant available nitrogen measured on rooted and non-rooted plots between $t = 0$ and $t = 24$ months ($n = 15$). Data points represent the average \pm SEs of all measurements made at the six rooted and non-rooted plots during one sampling occasion.

Rooting, total plant cover, plant species diversity, number of saplings, and height of saplings

Changes in soil properties induced by rooting significantly altered some of the vegetation properties monitored over the four years of the study. Total plant cover was significantly lower on the rooted plots compared to the non-rooted plots ($t = -2.15$, $p = 0.040$) but did not significantly vary between different sampling dates ($t = -1.62$, $p = 0.117$; Figure 5). The same pattern was found for the number of saplings (rooting: $t = -2.49$, $p = 0.019$; time: $t = -1.10$, $p = 0.281$; Figure 6). In contrast, we could not detect a rooting effect on sapling height ($t = -0.26$, $p = 0.798$). But sapling height varied significantly between different sampling dates ($t = -2.34$, $p = 0.027$), indicating normal plant growth over time (Figure 7). Finally, plant species diversity (Figure 8) showed no significant rooting effect ($t = 0.59$, $p = 0.557$) and also no significant difference between the different sampling dates ($t = 0.62$, $p = 0.553$). In general, plant species diversity and richness were rather low on both rooted and non-rooted plots (averaged over the 3 sampling events, we found only 5.03 different species on rooted plots and 5.00 different species on non-rooted plots).

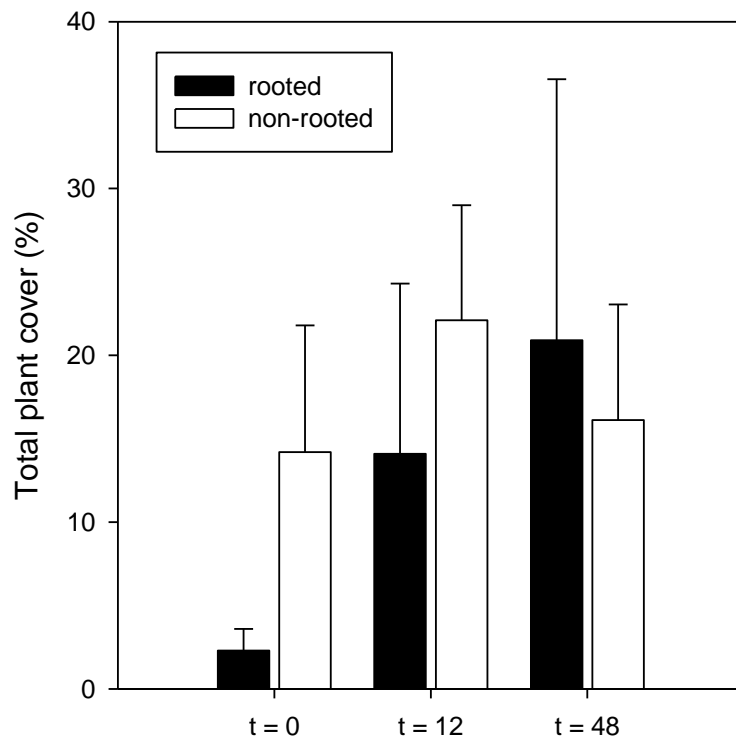


Figure 5: Total plant cover measured on rooted and non-rooted plots at time = 0, 12, and 48 months, respectively ($n = 6$ for all graphs). Data represent averages \pm SEs of sites.

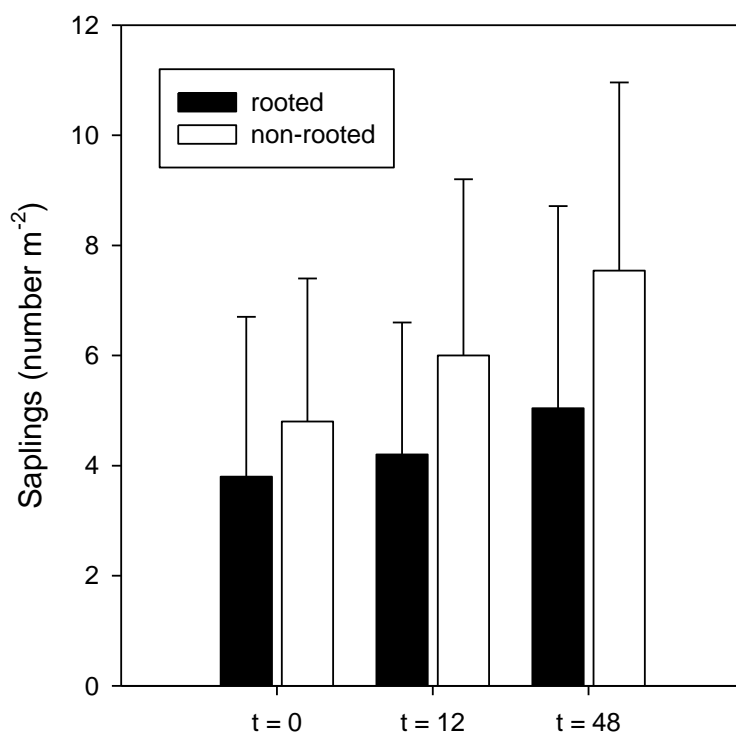


Figure 6: Number of saplings measured on rooted and non-rooted plots at time = 0, 12, and 48 months, respectively ($n = 6$ for all graphs). Data represent averages \pm SEs of sites.

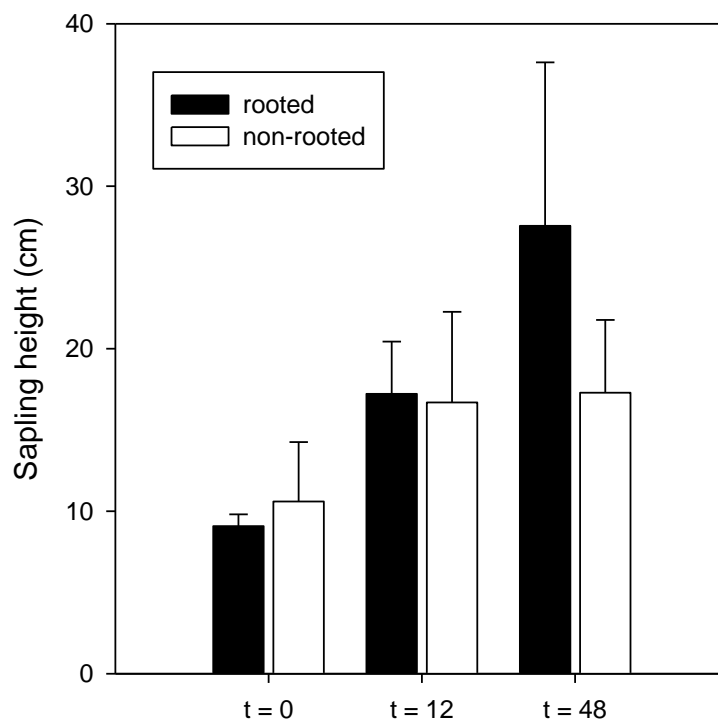


Figure 7: Sapling heights measured on rooted and non-rooted plots at time = 0, 12, and 48 months, respectively ($n = 6$ for all graphs). Data represent averages \pm SEs of sites.

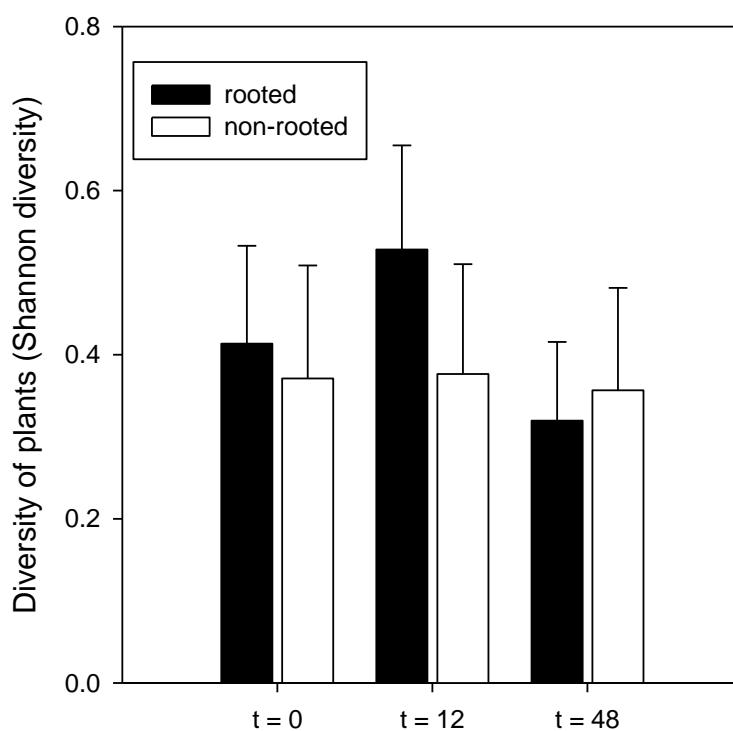


Figure 8: Diversity of plants measured on rooted and non-rooted plots at time = 0, 12, and 48 months, respectively ($n = 6$ for all graphs). Data represent averages \pm SEs of sites.

Discussion

Our results showed that rooting by wild boars was associated with higher soil C and N concentrations and microbial biomass C. Together with the elevated CO₂ emissions detected on rooted plots during the study (Risch et al. 2010), these findings suggest enhanced decomposition and faster turnover rates (mineralization) of C in rooted soils. Similar findings were reported by Lacki and Lancia (1983), who found higher OM concentrations in rooted compared to non-rooted plots in the Great Smoky Mountains National Park (GSMNP, USA). The authors explained their findings by stimulated OM decomposition on boar-rooted sites. In contrast, in other studies (Groot Bruinderink and Hazebroek 1996; Moody and Jones 2000; Mohr et al. 2005) no differences in OM, C and N concentrations were found between rooted and non-rooted plots. Given the higher turnover rates detected after boar rooting on our study sites, we were surprised that plant available N was not elevated, but significantly lower on the rooted compared to the control plots. These results stand in contrast to findings by Singer et al. (1984) who reported higher NO₃⁻-N and NH₄⁺-N concentrations on rooted plots indicating alteration in N transformation processes in GSMNP (USA). We can think of three potential explanations for the reduced N availability on the rooted compared to the non-rooted plots in our study, which we discuss below:

1. Nitrogen removal by plant uptake. Increased N uptake, storage or recycling by the vegetation could be responsible for the lower plant available N. Yet, in our study we did not detect higher plant growth; instead, total plant cover and the number of saplings were reduced on the rooted plots. Thus, alterations of understory N uptake likely cannot explain the lower availability of N. However, it is possible - as suggested by Singer et al. 1984 - that N was taken up by tree roots and tree growth was accelerated on rooted plots, as shown Lacki and Lancia (1986), who found greater shoot elongation of beech trees growing on rooted plots. Because we did not assess tree growth in our study, we cannot determine whether this mechanism would explain the lower N in our study.

2. Immobilization by microorganisms. It is possible that plant available N was immobilized by microorganisms on our rooted plots. Rooting by wild boars incorporates OM from the litter layer into the mineral soil (Groot Bruinderink and Hazebroek 1996), which increases resource availability (OM and C) and therefore favors the growth of the microbial communities. Since the C/N ratio of the incorporated OM is relatively high (typical for leaf litter), the soil microbes scavenge the soil solution to obtain enough N, which is required for the balance of nutrients (Homyak et al. 2008). Consequently, the increased microbial biomass found in our study could indicate that N was immobilized (incorporated into microbial cells), which

resulted in depleted soil N (Marhan et al. 2010). In contrast to our results, investigations conducted in steep oak forests in Germany revealed lower microbial biomass on rooted compared to non-rooted loamy soils (0 - 5cm; Mohr and Topp 2001; Mohr et al. 2005). These investigators found generally lower contents of organic C and total N in rooted plots compared to their control plots, which may explain the lower microbial biomass they found at their rooted plots. We are not aware of any other study assessing the effect of wild boars on soil microbial biomass.

3. Loss by leaching and erosion. Nutrients may have been leached and lost from the soil. Since the understory vegetation, especially the herbaceous layer, is often reduced on heavily rooted soils (e.g. Bratton, 1974, 1975; Peart and Patten 1992; Siemann et al. 2009) – which was also found in our study - nutrient uptake might be greatly reduced for some time after rooting (Singer et al. 1984). Thus, leaching of mineral N may be higher on rooted plots compared to plots with an intact herbaceous layer. In particular, due to their negative charge, nitrate ions are not adsorbed by the negatively charged colloids that dominate most soils (Gundersen et al. 2006). Therefore, nitrate ions move freely downward with drainage water and are readily leached. Moreover, by reducing soil bulk density, boar rooting may also accelerate soil erosion and therefore affect nutrient leaching directly. Supporting this idea, Mohr and Topp (2001) explained the decreases in organic C and total N of boar rooted soils in very steep oak stands as resulting from soil erosion and therefore loss of some of the freshly rooted material.

In addition to the impact on N availability, boars may influence understory vegetation directly by foraging (seed predation), uprooting, or mechanically damaging plants, therefore inhibiting certain plant species while favoring others. Yet, rooting had no effect on plant species diversity at our study sites. Similar to our findings, Bratton (1975) reported no effect on understory plant diversity. Several other studies found differences in plant species composition, but most of them focused on the impact of feral pigs in forests where they have been introduced (Aplet et al. 1991; Peart and Patten 1992; Siemann et al. 2009; Cuevas et al. 2010) or in grassland ecosystem (Kotanen 1995; Hone 2002; Cushman et al. 2004; Table 1). Where they are an invasive species, however, boars may affect the diversity and functioning of ecosystems differently and more extensively way than where they are native (e.g., Siemann et al. 2009). Authors that studied wild boars in their native range found increased species richness after rooting in conifer forest in Sweden (Welanders 1995) and in lowland rain forest in Malaysia (Ickes et al. 2001).

Overall, the net effect of disturbance by wild boars on understory vegetation in our study is complex and rather difficult to interpret. Yet, with the results of this present study, taking into account above- and belowground effects of rooting, we are able to depict a more complete picture of the role boars play in forest ecosystems. Obviously, by rooting the soil, wild boars may enhance decomposition and accelerate turnover rates (mineralization), but on the other hand also diminish plant available nitrogen, reduce total plant cover, and reduce sapling counts. Thus, "friend-or-foe" thinking in wild boar/forest debates must be handled with care. Consequently, this study contributes to the growing understanding of animal-plant, animal-soil and plant-soil interactions in forest ecosystems, which can serve to address ecosystem stability/resilience, biodiversity, and sustainable management issues. This knowledge will be valuable given the potential increases in wild boar populations and habitat range predicted for Switzerland (Geisser and Reyer 2004) and other European countries.

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Paper IV

Grubbing by wild boars (*Sus scrofa* L.) and its impact on hardwood forest soil carbon dioxide emissions in Switzerland

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Abstract

Interest in soil carbon storage and release has increased in recent years. In addition to factors such as climate/land-use change, vertebrate animals can have considerable impact on soil carbon dioxide (CO₂) emissions. To date, most research considered herbivores, while the impact of omnivorous animals has rarely been investigated.

Our goal was to determine how European wild boars (*Sus scrofa* L.), large omnivores that consume soil-inhabiting animals and belowground plant parts by grubbing in the soil, affect soil carbon dynamics. We measured soil respiration (CO₂), temperature, and moisture on paired grubbed and non-grubbed plots in six hardwood forest stands for a three-year period and sampled fine root and microbial biomass at the beginning and after two years of the study. We also measured the percentage of freshly disturbed forest soil within the larger surroundings of each stand and used this information together with hunting statistics and

forest cover data to model the total amount of CO₂ released from Swiss forest soils due to grubbing during one year.

Soil CO₂ emissions were significantly higher on grubbed compared to non-grubbed plots during the study. On average 23.1% more CO₂ was released from those plots, which we associated with potential alterations in CO₂ diffusion rates, incorporation of litter into the mineral soil and higher fine root/microbial biomass. Thus, wild boars considerably increased the small-scale heterogeneity of soil properties.

Roughly 1% of Switzerland's surface area is similar to our sites (boar density/forest cover). Given the range of forest soil disturbance of 27-54% at our sites, the GIS-model predicted that boars would release an additional 49731.10 to 98454.74 t CO₂ year⁻¹. These values are relatively small compared to total soil emissions estimated for Swiss hardwood forests suggesting boars having little effect on large-scale emissions unless their numbers increase and their range expand dramatically.

Keywords

Soil mixing, microbial biomass, fine root biomass, omnivore, GIS model

Introduction

Soils are an important component of the global carbon (C) cycle because they store over two-thirds of the terrestrial ecosystems C (Schulze 2006). Consequently, interest in understanding the factors that control this pool is high. Mineral soil C turnover rates and, therefore, the amount of carbon dioxide (CO₂) and other greenhouse gases released from the soil to the atmosphere have been shown to be a function of soil i) organic matter quality and quantity, ii) micro-climate (temperature, moisture), iii) physical properties (texture, bulk density), iv) chemical properties (pH, C/nutrient concentrations), and v) biological properties (microbial biomass, composition, diversity, activity; e.g., Kirschbaum 1995; Lomander et al. 1998; Epstein et al. 2002). Thus, any alteration of one or several of those parameters can increase or decrease soil CO₂ emission and organic matter decomposition rates, and the subsequent total C stored in soil.

Vertebrate animals can directly or indirectly alter soil biotic and abiotic properties (see e.g., reviews by Jefferies et al. 1994; Bardgett and Wardle 2003; Wardle et al. 2004; Pastor et al. 2006). By foraging on plant material they can induce changes in plant properties (C exudation, tissue nutrient content, plant community composition and productivity) and indirectly affect soil physical, chemical and biological properties as well as alter the quality

and quantity of organic matter that enters the soil (see e.g., Bardgett and Wardle 2003; Pastor et al. 2006). Additionally, they can directly affect soil biotic and abiotic properties through physical disturbance (e.g., Tardiff and Stanford 1998; van der Wal et al. 2007), by returning nutrients to the soil through waste (urine, feces) or carcasses (e.g., Carter et al. 2007; Fornara and du Toit 2008), and by altering the belowground community composition of microbes, invertebrates, and vertebrates through consumption (e.g., Russell and Detling 2003; Mohr et al. 2005). As a consequence, animals are likely to have a strong effect on the amount of C released from the soil as CO₂.

Most field studies on vertebrate-soil CO₂ interactions have been conducted in ecosystems inhabited by domestic (e.g., Morris and Jensen 1998; Rochette et al. 2000; Bremer and Ham 2002; Owensby et al. 2006; Jia et al. 2007), and to a lesser extent, native herbivores (e.g., Bremer et al. 1998; Knapp et al. 1998; Johnson and Matchett 2001; Risch and Frank 2006, van der Wal 2007). In contrast, surprisingly little research has focused on how omnivorous vertebrates (consuming both plants and prey) affect soil properties and subsequent CO₂ emissions. Although these animals consume less aboveground plant material compared to herbivores and have lower indirect effects on soil processes, they could potentially have a considerable direct effect on the soil system through 1) the physical disturbance of the soil when searching for forage (turning over the soil or litter layer) and 2) the alterations of the belowground community when consuming belowground plant parts or animals.

Wild boars (*Sus scrofa* L.) are omnivorous animals that obtain a considerable proportion of their diet by grubbing in the soil searching for plant seeds, roots/bulbs, vertebrate and invertebrate animals (= rooting; Howe and Bratton 1976; Wood and Roark 1980; Bratton et al. 1982; Baber and Coblenz 1987; Hone 1988). Grubbing involves breaking through the vegetation and typically mixes the top 15 cm to 70 cm of the litter and mineral soil (Imeson 1977; Genov 1981; Kotanen 1995). Consequently, wild boars, like other bioturbators, strongly affect soil physical, chemical and biological properties (review in Gabet et al. 2003; Singer et al. 1984; Lacki and Lancia 1986; Groot Bruinderink and Hazebroek 1996; Moody and Jones 2000; Sierra 2001; Mohr et al. 2005), and have the potential to exert a considerable impact on the amount of CO₂ released from the soil.

Wild boars are synanthropic species with a high reproductive potential, a high adaptability to a wide variety of habitats and a high ecological plasticity (Taylor et al. 1998). They naturally inhabited northern Africa, Europe and the whole of Asia, but also were introduced into the Americas, Australia and New Zealand. In many European countries, their population size has increased considerably during the past three decades and the boars have re-colonized regions

where they were drastically reduced or locally extinct during the 18th and 19th century (Schley et al. 1998; Geisser and Reyer 2005; Hebeisen et al. 2007). Several factors are likely responsible for those changes in boar numbers and distributions: i) higher frequency in mast years of beech and oak due to air pollution and environmental stress, ii) higher survival rates of piglets due to climate change related increased temperatures during winter, iii) increased agricultural use of corn as a crop, iv) increased size of agricultural fields (less disturbance, lower detectability of boars), v) trend in forest management towards replacing conifers with hardwoods, vi) baiting or diverting boars with corn feed by hunters and game managers, and vii) decreasing numbers or the elimination of natural predators (Feichtner 1998; Krüger 1998; Hespeler 2004; Melis et al. 2006). Today, European wild boar densities are estimated to range between 0.3 and 12.0 boars per km² (Melis et al. 2006; Hebeisen et al. 2007). In Switzerland, the population size has been steadily increasing since the 1980's, but the animals mostly occur in the northern- and southern-most areas of the country, where hardwood forests predominate. The primary objective of our study is to assess how grubbing by wild boars affects soil CO₂ emissions in hardwood forests in the Swiss central plateau. We measured soil CO₂ emissions, soil temperature and soil moisture over three years as well as determined fine root and microbial biomass in grubbed and non-grubbed soil at the beginning and after two years of the study to assess the impact these animals have on those properties. Further, we estimated total percentage of freshly disturbed forest soil by boars in our different forest stands twice during the study and used this information together with Swiss wild boar hunting statistics and forest cover data to estimate the effect wild boars might have on soil CO₂ emissions at the national scale using a Geographic Information System model.

Materials and Methods

Study area and sampling design

The study was conducted in hardwood and mixed hardwood forests located north of Zurich (47° 23' N and 8° 31' E) in the state of Zurich, Switzerland. The mean annual temperature is 9.2 °C, and the mean annual precipitation is 1137 mm (30 year averages from 1978 to 2008) with roughly 25% falling as snow during the months of November through February (MeteoSchweiz). We selected six 5 ha sites in beech-dominated (*Fagus sylvatica* L.) stands that also contained other hardwoods such as oaks (*Quercus* spp) and hornbeam (*Carpinus betulus* L.) and conifers (Norway spruce [*Picea abies* L. Karst]; Scots pine [*Pinus sylvestris* L.]). All sites were classified as typical *Galio oderati-Fagetum* following Keller et al. (1998) and were located within 50 km of each other. Elevation differences between our study sites

did not exceed 120 m. Soil physical and chemical properties were also similar among the sites (Table 1): The soil textural class was sandy loam underlain with sandstone parent material. The litter layer averaged 3 cm, rock content was 33%, and soil depths extended to 75 cm. Soils on these study sites were highly acidic (all pH's less than 4.0).

Table 1: Elevation, soil chemical and soil physical properties of the top 45 cm of the mineral soil of the six sites under study (data from non-grubbed plots established in May 2006).

Site	Elevation (m)	pH	Carbon (%)	Nitrogen (%)	Soil bulk density (g cm ⁻³)	Soil particle size distribution			Soil textural class
						Sand (%)	Silt (%)	Clay (%)	
1	550	3.18	2.16	0.13	1.06	72	18.1	9.9	Sandy loam
2	550	3.55	3.1	0.17	1.14	76.3	13.4	10.3	Sandy loam
3	560	2.92	3	0.14	1.01	74.6	14.4	11	Sandy loam
4	610	2.93	4.05	0.17	1.04	69.1	20.3	10.6	Sandy loam
5	650	2.91	3.6	0.17	1.02	66.2	23.8	10	Sandy loam
6	670	2.81	5.05	0.22	0.83	68.2	22.5	9.3	Sandy loam

Extensive grubbing events larger than 100 m² in size originating from grubbing between October 2005 and February 2006 were detected at all sites in spring 2006. We established a paired plot design consisting of a grubbed (disturbed) and non-grubbed (undisturbed) plot 10 x 10 m in size at each study site in late May 2006. The non-grubbed plot was randomly selected in close proximity to the grubbing incident, but located at least 15 m from the grubbed plot. Since grubbing generally is visible for roughly three years in the forests we worked in, our non-grubbed plots have, if ever, not been disturbed for three years. Both the grubbed and non-grubbed plots were fenced with 1.3 m high knotted mesh (Ursus 130/11/15; mesh size at 0-50 cm height = 10 x 15 cm; mesh size at 50-110 cm = 15 x 15 cm; mesh size at 110-130 cm = 20 x 15 cm; Hortima AG, Hausen, Switzerland) tightened around 1.6 m square wooden posts (15 x 15 cm) to prevent boars from entering. Two 2.5 mm diameter wires were affixed at 1.4 and 1.5 m height to prevent animals (roe deer) from jumping over the 1.3 m mesh.

To determine if boars specifically select foraging sites within a homogeneous stand or randomly grub in the soil, we established a third randomly chosen, non-grubbed and non-fenced 10 x 10 m plot at each study site at the beginning of the study (May 2006). All these plots were subject to grubbing until the end of the study and, therefore, we assume that boars do not specifically select the sites where they are grubbing. Thus, our randomly chosen non-

grubbed fenced plots were sufficient controls to be compared with the respective grubbed plots. Further, a fourth plot was established at each site in June 2008. These plots were not disturbed between October 2005 and October 2007, but were freshly grubbed between October 2007 and March 2008. These plots were used to determine if grubbing at different times at different plots would produce the same soil CO₂ efflux data as those study plots chosen in 2006.

Soil sampling and soil CO₂ emission measurements

Soil samples were collected twice. The first set of samples was collected at both the grubbed and non-grubbed plots just after plot establishment in early June 2006. The second soil sampling date was two years after plot establishment in early June 2008. Each time we randomly selected six locations in each plot to collect mineral soil samples using a 5 cm diameter core sampler. Samples were taken at 15 cm depth intervals to a total depth of 45 cm, after removing the litter layer. Soil samples were collected at least one meter from the fence to avoid edge effects. Three of the six samples were dried at 65 °C and fine roots were manually separated from the soil material to obtain fine root biomass (dry weight). The other three samples were immediately put on ice, taken to the laboratory, sieved through a 2 mm sieve and stored for two weeks in the cold room at 4 °C. Microbial biomass was then determined using substrate-induced method of Anderson and Domsch (1978). De-ionized water was added as needed to bring all samples to 60% water-filled pore space. The samples were then incubated at room temperature for 10 days prior to analyses to subside spurious microbial activity associated with the sampling disturbance. This incubation period was determined in a preliminary experiment as a minimum before microbial activity declined to a steady state (basal respiration). Microbial biomass was determined using 25 g of mineral soil (dry-weight equivalent) and a glucose concentration of 5 g kg⁻¹ soil. CO₂ production was analyzed 1 to 2 hours following the addition using a LI-COR 6200 gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

In situ soil CO₂ emissions were measured with a PP-Systems SRC-1 soil respiration chamber (closed circuit) attached to a PP-System EGM-4 infrared gas analyzer (PP-Systems, Hitchin, Hertfordshire, UK) on five randomly selected locations within each grubbed and non-grubbed plot. For each measurement the soil chamber (15 cm high; 10 cm diameter) was placed on permanently-installed PVC collars (10 cm diameter) driven five centimeters deep into the mineral soil at the beginning of the study. The measurements were conducted between 0900 and 1700 hrs every six weeks (except between November 2006 and March 2007 due to

broken analyzer) starting in June 2006 and ending in June 2008 (total number of measurements = 15). In addition, we measured soil CO₂ emissions every six weeks on the freshly grubbed plots (installed in June 2008) and non-grubbed plots (installed in May 2006) from July 2008 to May 2009 (no measurements conducted in December and January due to snow on the ground). Freshly germinated plants growing within the PVC collars were removed prior to each of the measurements. We measured soil temperature with a waterproof digital pocket thermometer (Barnstead International, Dubuque IA, USA) and soil moisture with a field-scout TDR-100 (time domain reflectometer; Spectrum Technologies, Plainfield IL, USA) for the 0 to 10 cm mineral soil depth at five random points at each plot during each of the soil CO₂ emission measurements.

Statistical analyses

To investigate whether soil CO₂ emissions, soil temperature and moisture differed between the paired grubbed and non-grubbed plots between June 2006 to June 2008 we used a Linear Mixed Model procedure in order to avoid potential pseudoreplication of the data: Soil CO₂ emission, soil temperature and soil moisture, respectively, were a function of the two fixed-effects treatment (grubbed and non-grubbed) and time and the random-effect site. We also adjusted these models for temporal autocorrelation where necessary. The same Linear Mixed Model approach was used to assess the differences in soil CO₂ emission between the originally installed non-grubbed (May 2006) and the freshly grubbed (June 2008) plots. Soil temperature and moisture were highly correlated for both the grubbed and the non-grubbed plots. We therefore tested whether the residuals of this model helped to further explain any of the remaining variance before assessing the relationships between soil moisture or soil temperature (independent variables) and soil CO₂ emissions (dependent variables) with linear regression analyses. Differences in fine root and microbial biomass between the grubbed and non-grubbed plots were tested using two-tailed paired t-tests for both sampling dates. Alpha-level was 0.05 for all analyses. The normality and homogeneity criteria were met for all data, thus we did not transform our data.

Extrapolation of the effects of boar grubbing on soil CO₂ emissions

Percentage of grubbed soil area in the forest stands surrounding our sites

We established four 200 m long and 1 cm wide (width of measuring tape) transects at each of our six sites to determine the percentage of freshly grubbed soil surface area at our sites (from previous winter until spring). We chose the northern-, eastern-, southern-, and western-most

point of each paired plot as transect starting points. The extent of fresh grubbing along each transect was recorded and the percentage of the transect that was disturbed was calculated. We then averaged the percentages of all four transects per site to obtain an estimate of the total percentage of grubbed forest soil. The inventory was conducted when the study was established in spring 2006 and repeated in spring 2008. From these two inventories we calculated an average percentage of fresh grubbing events for our study sites.

Estimating forest area inhabited by wild boars

Population counts of wild boars are not available for Switzerland. To estimate wild boar densities we used the average number of wild boars culled during hunting seasons 2003 to 2006 (hunting statistics) provided by state agencies for each county or hunting district of the respective state (depending on hunting regimes of the respective state; 2275 units in total). From these statistics we calculated the number of wild boars culled per 10 km² for each county or hunting district and entered the information into ArcGIS 9.2. Since our six study sites were located in areas where on average more than 10 boars per 10 km² were culled between 2003 and 2006, we merged all counties/hunting districts for entire Switzerland with 10 or more boars culled per 10 km² to a new layer. We then performed a spatial join of this layer with an existing GIS layer containing information on forest cover type (provided by the Swiss Federal Statistical Office 2010): Hardwood and mixed-hardwood were defined as potential wild boar habitat, mixed-coniferous and coniferous as non-boar habitat. The resulting joined layer allowed us to calculate the total area of Switzerland that we considered to be potentially inhabited by wild boars in similar density to our sites ("boar areas"). We assumed that those sites were similarly affected by boars as our sites.

Effect of wild boars on soil CO₂ emission at large scales

To estimate the amount of CO₂ released from Swiss forest soils as a result of wild boar grubbing, we calculated the average difference in CO₂ emissions between our grubbed and non-grubbed plots using all measurements conducted during the first year of measurement (June 2006 through June 2007). We assumed that the differences in CO₂ emissions between grubbed and non-grubbed plots remained constant over the course of the day since the CO₂ emissions and temperatures measured showed a strong linear relationship. We then multiplied the potentially grubbed area from the "boar areas" layer with the percentage grubbed area from the disturbance inventory to estimate the area of grubbed forest soil of the "boar areas". The area of grubbed forest soil was then multiplied by the average difference in CO₂ emissions between the grubbed and the non-grubbed plots and extrapolated to a yearly basis.

Results

Grubbing effects on soil properties

In these sandy loam soils, CO₂ emissions ranged from 0.06 to 0.52 g CO₂ m⁻² h⁻¹ on the grubbed plots and from 0.04 to 0.43 g CO₂ m⁻² h⁻¹ on the non-grubbed plots between June 2006 and June 2008. The rates were significantly higher on the grubbed (average \pm standard error for the entire time of study: 0.30 ± 0.04 g CO₂ m⁻² h⁻¹) compared to the non-grubbed plots (0.24 ± 0.04 g CO₂ m⁻² h⁻¹) throughout the entire time of measurement (No. of measurements taken over time (time) = 15, $t = -5.058$, $p < 0.001$; Figure 1a). On average 0.06 g CO₂ m⁻² h⁻¹ or 23.1% more CO₂ was released from grubbed plots during these two years of measurement. We also found on average 0.075 g CO₂ m⁻² h⁻¹ higher emissions on the freshly grubbed (winter/spring 2007/2008) compared to the non-grubbed plots between July 2008 and May 2009 (time = 7, $t = -3.043$, $p = 0.003$). Thus, regardless of the location within the site, grubbing resulted in similar differences in CO₂ emissions between the grubbed and non-grubbed plots.

Between June 2006 and June 2008 soil temperature ranged from 4.1 to 17.5 °C at all the sites, but did not significantly differ between the grubbed (average: 10.97 ± 1.18 °C) and the non-grubbed (10.87 ± 1.17 °C) plots [time = 14 (one missing measurement due to broken thermometer), $t = -0.815$, $p = 0.416$; Figure 1b]. Soil moisture ranged from 7.0 to 24.3% ($14.1 \pm 0.72\%$) on the grubbed and from 6.7 to 26.3% ($14.9 \pm 0.72\%$) on the non-grubbed plots. Soil moisture values were significantly lower for the grubbed compared to non-grubbed plots [time = 14 (one missing measurement due to broken TDR), $t = 2.078$, $p = 0.039$; Figure 1c].

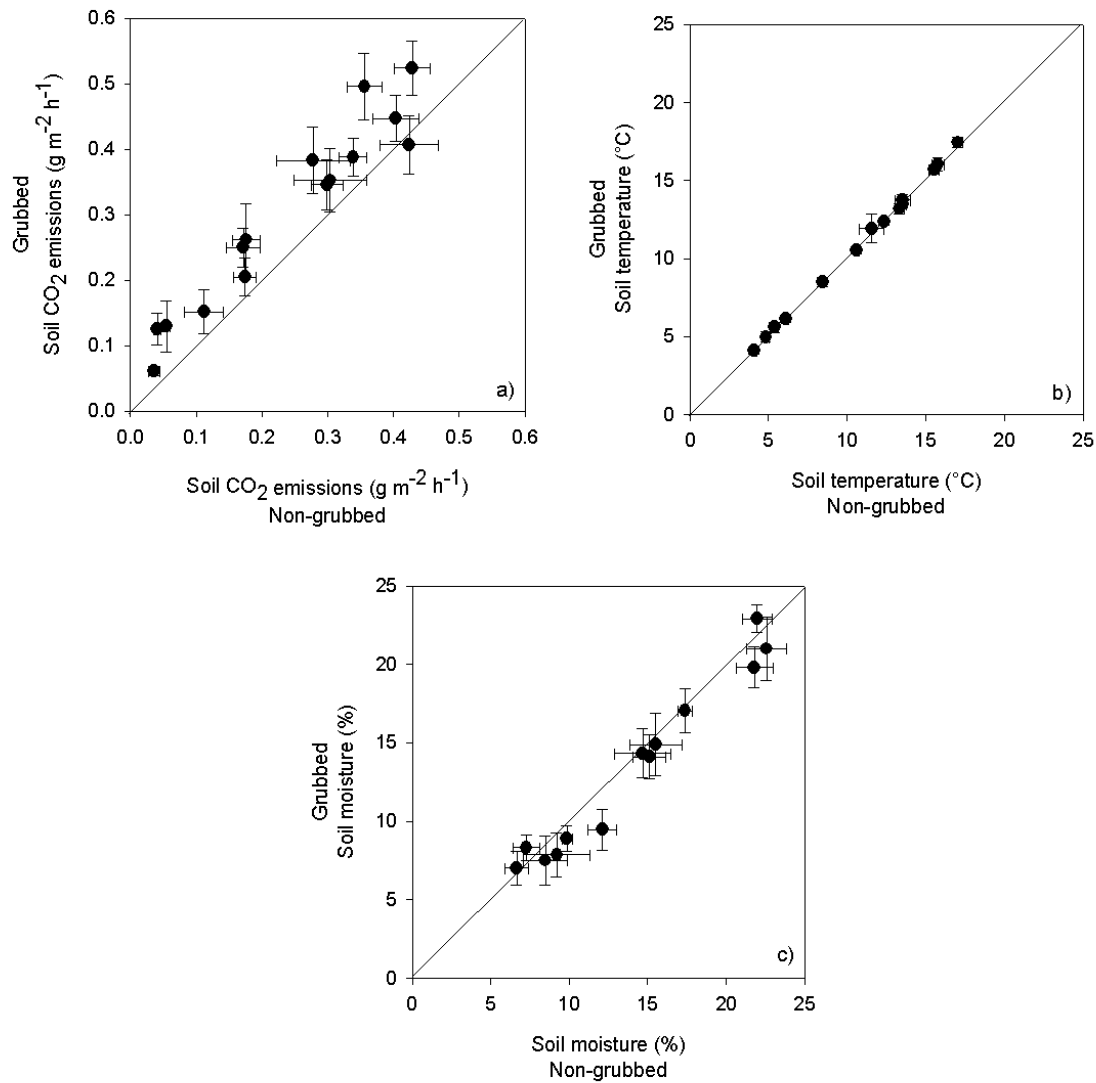


Figure 1: Soil CO₂ emissions, soil temperature and soil moisture measured on grubbed and non-grubbed plots between June 2006 and June 2008: a) soil CO₂ emissions in g m⁻² h⁻¹; b) soil temperature in °C; c) soil moisture in %. A data point represents the average \pm standard error of all the measurements collected at the six grubbed and non-grubbed plots, respectively, during one sampling occasion (time) between June 2006 and June 2008.

Soil temperature and moisture were both highly correlated at both the grubbed and the non-grubbed plots with soil temperature having a negative effect on soil moisture (grubbed: $y = -1.105x + 26.238$, $r^2 = 0.638$, $df = 13$, $F = 21.124$, $p = 0.001$; non-grubbed: $y = -1.154x + 27.479$, $r^2 = 0.630$, $df = 13$, $F = 20.439$, $p = 0.001$, where x is soil temperature and y soil moisture; Figure 2a). When plotting the residuals of these two models against soil CO₂ emissions, it became evident that adding in both soil temperature and moisture would not improve the model (Figure 2b). Since soil temperature likely is driving soil moisture in the stands under study and not vice-versa, we analyzed the relationship between soil temperature and soil CO₂ emissions. These variables showed a strong positive relationship for both the

grubbed and the non-grubbed plots (grubbed: $y = 0.032x - 0.037$, $r^2 = 0.894$, $df = 13$, $F = 101.448$, $p < 0.001$; non-grubbed: $y = 0.030x - 0.082$, $r^2 = 0.830$, $df = 13$, $F = 58.690$, $p < 0.001$; Figure 2c). Soil CO₂ emissions, soil temperature, and soil moisture all varied significantly over the course of the study (p -values < 0.001). Soil CO₂ emissions and temperature showed higher values during the summer and lower values during the winter months, while soil moisture showed the opposite pattern (higher in winter, lower in summer). Because our sites were sandy loam textured, the study plots were never saturated to result in anaerobic conditions.

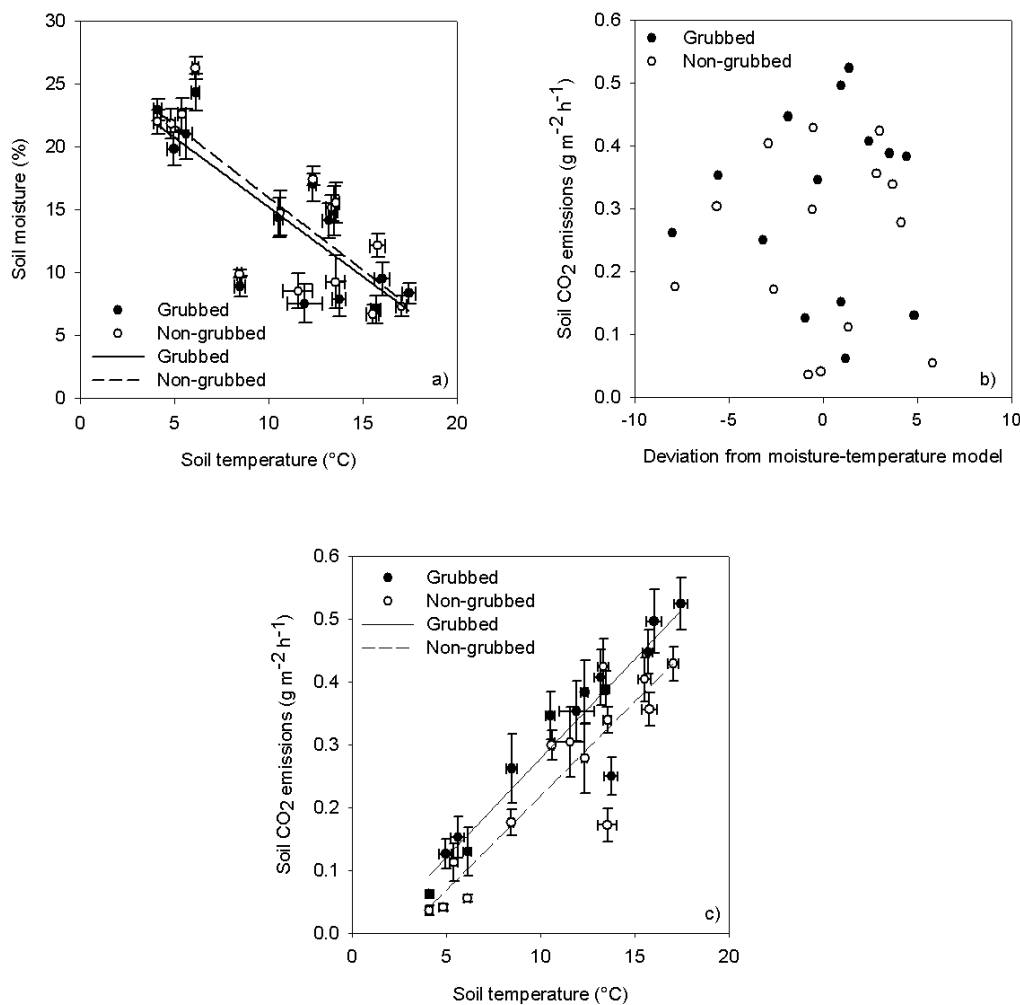


Figure 2: Relationships between soil CO₂ emissions, soil temperature and soil moisture measured on grubbed and non-grubbed plots between June 2006 and June 2008: a) Relationship between soil temperature and moisture; b) Relationship between deviation from soil temperature-moisture model and soil CO₂ emissions; c) Relationship between soil temperature and soil CO₂ emissions. All regressions are linear. A data point represents the average \pm standard error of all the measurements collected at the six grubbed and non-grubbed plots, respectively, during one sampling occasion (time) between June 2006 and June 2008.

Fine root biomass in the top 45 cm of the mineral soil averaged $0.006 \pm 0.001 \text{ g cm}^{-3}$ soil (dry weight) for grubbed plots in June 2006 and was significantly higher than the $0.004 \pm 0.001 \text{ g cm}^{-3}$ soil we found in the non-grubbed plots ($df = 5$, $t = 4.27$, $p = 0.007$; Figure 3a). After two years, fine root biomass was similar at the grubbed ($0.003 \pm 0.0003 \text{ g cm}^{-3}$ soil) compared to the non-grubbed plots ($0.002 \pm 0.001 \text{ g cm}^{-3}$ soil; $df = 5$, $t = 0.39$, $p = 0.39$; Figure 3a). A similar pattern was found for microbial biomass of the top 45 cm of the mineral soil: the values were significantly higher on the grubbed compared to the non-grubbed plots ($df = 5$, $t = 3.10$, $p = 0.03$), averaging $822.6 \pm 94.2 \text{ mg kg}^{-1}$ soil (grubbed) and $541.0 \pm 0.1 \text{ mg kg}^{-1}$ soil (non-grubbed), respectively, at the beginning of the study in June 2006 (Figure 3b). In June 2008 microbial biomass at the grubbed plots ($1235.2 \pm 154.2 \text{ mg kg}^{-1}$ soil) was no longer significantly different from the one at the non-grubbed plots ($841.5 \pm 86.7 \text{ mg kg}^{-1}$ soil; $df = 5$, $t = 1.85$, $p = 0.12$; Figure 3b). Our findings indicate that grubbing by wild boars had a long-lasting effect on soil CO_2 emissions, significantly increasing the small-scale spatial heterogeneity of this parameter. Fine root and microbial biomass were only significantly higher after the initial grubbing event. The reason of not finding significant differences in these two parameters two years after the initial grubbing event may be due to i) time since grubbing (incorporation of the litter layer into the mineral soil long time ago), ii) within plot variability of both fine roots and microbial biomass and/or iii) the small number of replicate paired plots ($n = 6$; see also Figure 3).

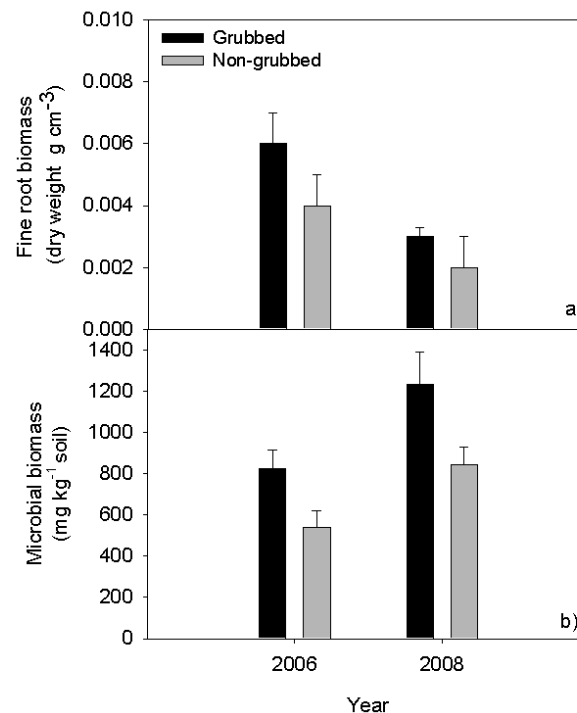


Figure 3: Fine root and microbial biomass on grubbed and non-grubbed plots measured in June 2006 and June 2008 ($n = 6$ for all graphs): a) fine root biomass in g cm^{-3} (dry weight) for the top 45 cm of the mineral soil; b) microbial biomass in mg kg^{-1} soil for the top 45 cm of the mineral soil. Data represents averages \pm standard errors of all sites.

Extrapolation of the effects of boar grubbing on soil CO₂ emissions

The percentage of disturbed forest soil averaged 53.8% in 2006 and 27.2% in 2008 at our sites. We used the 2006 values as maximum, the 2008 values as minimum and average of both (40.5%) for our calculations, resulting in three different disturbance scenarios. The total area of Switzerland with densities of culled boars larger than 10 animals per 10 km² and covered by hardwood or mixed hardwood forests amounted to 410 km² (Figure 4), which corresponds to roughly 1% of Switzerland's surface area. Based on these assumptions, we calculated the quantity of CO₂ released given the different disturbance conditions. Assuming 53.8% of the area as disturbed (maximum), 98454.74 t of CO₂ year⁻¹ in addition to the regular soil CO₂ emissions would be released by grubbing. If we assume 40.5% (average) of the forest soil to be disturbed, then the additional emissions would amount to 74092.91 t CO₂ year⁻¹, whereas 49731.10 t CO₂ year⁻¹ would be released given the minimum percentage (27.2%) of soil disturbance.

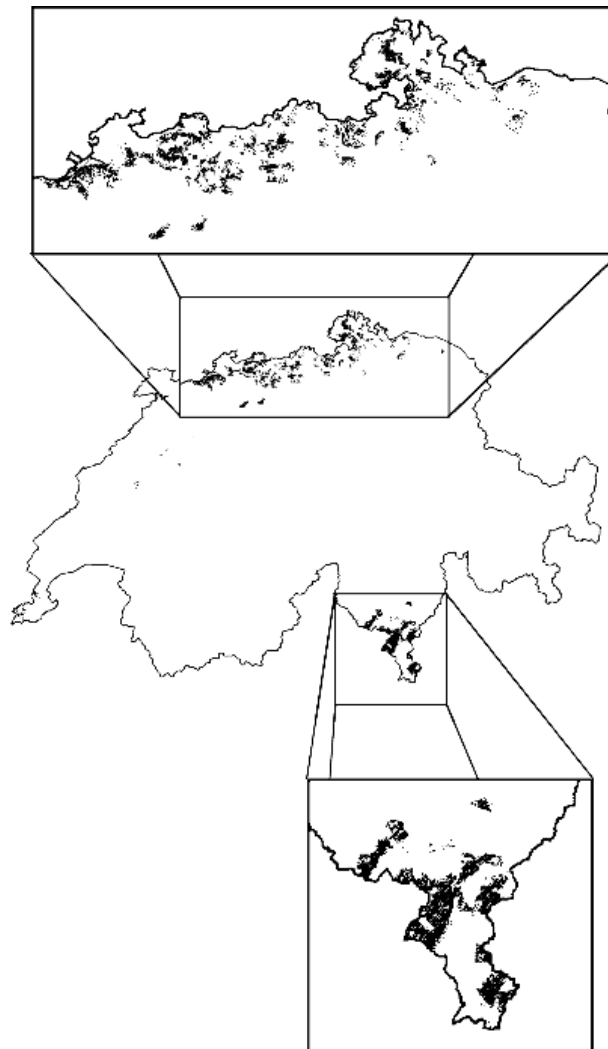


Figure 4: Areas within Switzerland where on average (2003 to 2006) more than 10 boars have been culled per 10 km² and where the forest cover type was classified as hardwood or mixed hardwood forest by the Swiss Federal Statistical Office (2010). Exerts show the northern- and southern-most parts of Switzerland, where the model predictions were highest.

Discussion

Grubbing effects on soil CO₂ emissions

Our results showed that grubbing by wild boars had a long-lasting effect on soil CO₂ emissions as we measured significantly higher emission rates on the grubbed compared to the non-grubbed plots throughout the study. We are not aware of any other study that has measured the effects of grubbing by wild boars or another omnivorous vertebrates on soil CO₂ emissions. However, similar to grubbing by boars, soil disturbance by pink-footed geese searching for belowground plant parts early in the season was found to have a strong effect on C dynamics in a tundra ecosystem reducing both photosynthesis and ecosystem respiration (van der Wal et al. 2007). Unfortunately, soil CO₂ emissions were not measured in this study, making a comparison difficult.

Forest management practices (site preparation) that mix the surface litter layer with the mineral soil also seem to have a similar effect on soil CO₂ emissions as grubbing by wild boars. For example, Mallik and Hu (1997) found over 50% higher soil CO₂ emissions on clear-cut plots when they mixed the soil to a depth of 20 cm compared to the uncut control stands. Similarly, Pumpanen et al. (2004) reported 20% higher soil CO₂ emissions from soils of clear-cut coniferous stands where the mineral soil and litter layer were mixed (mounding) compared to the clear-cut only plots. In addition, they found 32% higher soil CO₂ emission values on the clear-cut mounded plots compared to plots established in uncut control stands. However, the studies that assessed the effect of site preparation on soil CO₂ emissions have looked at clear-cut sites. These sites would likely respond somewhat differently than our sites grubbed by boars where no clear-cutting occurred and forest cover was maintained. Overall, the magnitude and seasonal variability of our soil CO₂ emission rates at the non-grubbed plots lay within the ranges of what has been reported from other hardwood forest ecosystems in Europe (e.g., Jonard et al. 2007; Knohl et al. 2008).

We can think of three explanations for the differences in CO₂ emissions that were detected between the grubbed and non-grubbed plots in our study. Moldrup et al. (2004) modeled soil gas diffusion as a function of air-filled porosity, total porosity and macro-porosity and demonstrated that drier soils have higher diffusion rates compared to wetter soils. Thus, it is possible that the decrease in soil moisture that we detected as a result of grubbing was directly responsible for the increases in soil CO₂ emissions. It also has been shown that a soil with decreased soil porosity, for example as a result of soil compaction, has lower diffusion rates than non-compacted soils (e.g., Conlin and van den Dierssche 2000; Shestak and Busse 2005). If the opposite would be true for soils that are “plowed” – i.e., that soils with higher porosity

have higher gas diffusion rates - then our higher soil CO₂ fluxes observed within the grubbed plots could also be related to increased soil porosity. A third explanation for our results would be that higher auto- and heterotrophic respiration, as a result of soil disturbance (bioturbation), were responsible for our findings, which we will discuss in detail in the following:

(1) Grubbing by wild boars is known to incorporate the litter layer into the mineral soil (e.g., Singer et al. 1984; Brownlow 1994), which increases the amount of C available for the decomposer food web. Thus, soil nutrient mobility, availability and mineralization rates and the amount of roots and microbes could be affected. Several studies have investigated soil carbon and nutrient availability and/or mineralization on grubbed and non-grubbed soil. They have found positive (Singer et al. 1984), neutral (Groot Bruinderink and Hazebroek 1996; Moody and Jones 2000; Cushman et al. 2004; Mohr et al. 2005; Tierney and Cushman 2006) or negative (Mohr and Topp 2001) effects of wild boar grubbing in oak/oak-beech forests and grassland ecosystems as well as positive effects of grizzly bear digging on ammonium-N and nitrate-N availability in subalpine meadows (Tardiff and Stanford 1998). Two of the studies listed above also provided data on microbial biomass (Mohr and Topp 2001; Mohr et al. 2005) showing lower values for grubbed compared to non-grubbed loamy soils of dry oak forests located in the western part of Germany. Potential reasons for these highly variable and in some cases contrasting results to our own findings could be related to differences in ecosystem properties (e.g., soil physical, chemical and biological properties, vegetation type), boar densities, or climatic conditions (air temperature, precipitation) among the studies.

(2) Besides integrating the litter into the mineral soil and increasing soil C available for microbes, grubbing by wild boars could have led to higher microbial biomass by decreasing the size of soil aggregates. Such decreases have been shown to occur after tilling and result in higher amounts of inter-aggregate unprotected light fraction organic matter, which directly favors microbial growth (Grandy and Robertson 2006a,b). Similarly, Goebel et al. (2009) showed in a laboratory trial with soils originating from both agricultural land and forests that intact soil macro-aggregates released less CO₂ than crushed material. Unfortunately, we could not find any study that would have assessed how wild boars or other animals affect soil aggregate size when grubbing. However, it is likely that they would have a similar effect as a tractor plowing the soil.

(3) Root growth has been shown to be higher on soils with lower bulk densities, compared to soils with higher bulk densities (Siegel-Issem et al. 2005). Grubbing by wild boars leads to decreases in soil bulk density because of the mixing of litter with the mineral soil (Singer et al. 1984) as we also found at our sites (unpublished data). Such decreases in bulk density

could directly favor root growth. In addition, the changes in bulk density could also alter the composition of the soil fauna. Such compositional changes could lead to higher root herbivory rates and therefore negatively affect root growth. Alternatively, alterations of the soil fauna could affect the availability of soil nutrients and indirectly change root growth. However, we are not aware of any study that conducted such an investigation. Microbial biomass, in contrast to root growth, has generally been suggested to be rather robust to changes in soil bulk density (e.g., Shestak and Busse 2005, Busse et al. 2006), thus microbial biomass probably was not altered as a result of decreased bulk density due to grubbing by wild boars.

(4) Consumption of soil-inhabiting vertebrates and invertebrates by boars (e.g., Genov 1981; Fournier-Chambrillion et al. 1995; Baubet et al. 2003) could affect the composition and abundance of root herbivores, bacterial feeders, other soil animals or fungi and therefore alter fine root and microbial biomass. For example, Mohr et al. (2005) reported significantly lower numbers of saprophagous and predatory soil arthropods as well as isopods, while the species numbers of the Araneae family (spiders) were not affected in plots subject to simulated grubbing in oak stands in Germany. Mohr and Topp (2001) found decreases in numbers of soil arthropods where wild boars and red deer (*Cervus elaphus* L.) had disturbed the soil. Similar to these results wild pigs inhabiting Hawaiian forests significantly reduced both the abundance and biomass of soil micro-arthropods. However, they had no effect on nematodes and larger soil invertebrate animals (Vtorov 1993). Even though there is strong evidence of shifts in the composition and abundance of soil inhabiting animals following grubbing by large mammals, there is no information available on how such changes affect soil microbial or root biomass.

(5) It is possible that wild boars deposit considerable amounts of dung and urine while searching the soil for forage. These additions of readily available nutrients to the forest soil could result – at least temporarily - in increased fine root and microbial biomass, and subsequent higher soil CO₂ emission rates where boars have grubbed. Such increases in root and microbial biomass have been studied in ecosystems where large herbivores occur in high densities (e.g., Frank and Evans 1997; van der Wal et al. 2004). Whether the same effects would be found in systems inhabited by wild boars, which generally occur in much lower densities compared to large herbivores, remains to be studied.

Based on all the evidence we found in the field we feel confident to assume that boars did grub randomly in the forests under study and that our randomly chosen non-grubbed fenced plots were sufficient controls to be compared with the respective grubbed plots. However, we cannot rule out that some of the differences in soil or plant properties that we measured

already existed prior to grubbing. Unfortunately, such differences could not be accounted for as it is not possible to predict where the boars are going to grub and therefore “pre-treatment” sampling was not possible.

Extrapolation of the effects of boar grubbing on soil CO₂ emissions

The percentage of forest soil disturbance caused by wild boars at our sites was approximately within the range reported by other studies conducted in hardwood stands. For example, Howe et al. (1981) found that roughly 80% of the surface area was disturbed in a beech forest in Tennessee, USA and Welander (2000) reported boar rooting frequencies ranging from 4% in dry soils to 75% in mesic soils in deciduous forest stands in Sweden. In contrast, less than 10% of the surface area was disturbed by grubbing in a montane rain forest in Hawaii, USA (Ralph and Maxwell 1984), or in Eucalyptus stands in Australia (Hone 1988).

When using the average percentage of freshly disturbed forest soils measured in our stands together with the approximation of boar densities and forest cover, we calculated that grubbing by wild boars would result in a release of 49731.10 to 98454.74 t additional CO₂ per year in Switzerland. These numbers likely underestimate the total effect boars have on forest soil CO₂ emissions as they neither account for patches that were grubbed in previous years nor do they consider sites inhabited by fewer boars compared to our sites. Overall, they only represent between 0.64 and 1.26% of the soil CO₂ emissions estimated for Swiss hardwood and mixed-hardwood forest soils (7802006.40 t CO₂ year) using our average soil CO₂ emission rate measured at the non-grubbed plots (0.24 g CO₂ m⁻² h⁻¹) and the estimated total of 3711 km² Swiss hardwood/mixed hardwood forest soils based on the National Forest Inventory (Brassel und Brändli 1999). However, even if we were able to include older grubbing events as well as all boar inhabited areas into our model, the contribution of boars grubbing to the total amount of CO₂ released from Swiss hardwood/mixed hardwood soils likely would remain rather negligible given current population sizes. Nevertheless, the contributions could increase if the animals increase in population size and if they extend their range within the country. To date they are confined to the northern and southern-most parts of Switzerland (cf. Hausser 1995) due to highways and mountain ranges that act as barriers. Yet, the animals are expected to overcome these obstacles eventually, thus, their impact on forest soil CO₂ emissions might increase in the future. Nevertheless, it also is possible that the C source-sink relationships of the forests the boars inhabit will remain unaltered due to increased tree growth on soils where they have grubbed. For example, Lacki and Lancia (1986) reported a positive relationship between shoot elongation of American beech and the

rooting of boars, which indicates that the amount of C lost from the soil through grubbing could be neutralized by increased photosynthetic uptake of CO₂ by the trees. Further investigations will show whether this would be the case at our study sites.

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Synthesis

Wild boar populations are growing from small, fragmented groups to larger sustained populations throughout northern Switzerland. Given the obvious disturbance to the forest soil by wild boar rooting (grubbing), it was hypothesized that wild boars influence disturbance-sensitive forest ecosystem properties. The main goal of this thesis therefore was to investigate how wild boar rooting impacts above- and belowground properties of mixed-hardwood forest stands, the most common type of wild boar habitat in Switzerland.

It is well known that rooting by wild boars incorporates the litter into the soil (Singer et al. 1984). The disintegration and incorporation of litter residues into the soil was shown to enhance decomposition rates, and lead to higher concentrations of OM, C, and N in the mineral soil horizons of rooted compared to non-rooted plots (Lacki and Lancia 1983). The same results were found in our study (Paper I, Figure 1, Table 2). Further, the ploughing-like foraging behaviour of wild boars reduced the soil bulk density by mixing air into the soil (Paper I, Table 3). Higher soil OM, C, and N concentrations and reduced soil bulk density were responsible for not finding any net rooting effect on OM, C, and N pools in the mineral soil (Paper I, Figure 2, Table 3). However, rooting was found to significantly alter CO₂ fluxes (Paper VI, Figure 1) and N mineralization rates (Paper III, Figure 4). The higher CO₂ fluxes on the grubbed plots were likely explained by higher fine root/microbial biomass, enhanced decomposition rates and faster turnover rates, as well as potential alterations in CO₂ diffusion rates.

Complementary to the soil chemical and physical components discussed above, Paper II focused on soil biological parameters. We expected that increased resource availability resulting from boar rooting (higher OM, C, and N concentration reported in Paper I, Figure 1, Table 2) would favour the growth of microbial communities. Microbial biomass C did not differ between the rooted and non-rooted plots for the 0 to 15 cm mineral soil depth (Paper II, Figure 3), but significantly higher microbial biomass was found when sampling the top 45 cm of the mineral soil (Paper IV, Figure 3). The only two other studies (Mohr and Topp 2001; Mohr et al. 2005) that have assessed the effects of wild boar grubbing on soil microbial biomass carbon revealed opposite findings compared to ours. They found lower values for microbial biomass carbon on rooted compared to non-rooted soils in steep oak forest plots in Germany. However, they also reported lower contents of organic C and total N at the rooted

compared to the control plots, supporting the idea of correlation between resource availability and bacterial communities mentioned above.

Changed environmental conditions caused by soil disturbance through wild boar rooting may also affect soil bacterial community structures. However, contrary to this hypothesis, we could not detect a statistically significant difference in mineral soil bacterial community structure or in the bacterial diversity indices (richness, Shannon Diversity, and evenness, Paper II, Figure 1, 2) between rooted and non-rooted plots. Thus, the bacterial communities responded with resistance to the soil disturbance by rooting. However, we found a clear seasonal pattern in microbial community structures (Paper II, Figure 1), which could have obscured the disturbance effect at our sites.

Given the effects of wild boar rooting on soil chemical, physical, and biological properties, we expected that rooting would also affect – directly through mechanical damage or indirectly via changes in soil properties – the forest understory vegetation (Paper III). Indeed, total plant cover and number of saplings were reduced on rooted plots (Paper III, Figure 5, 6). In contrast, rooting did not alter tree sapling height and plant species diversity (Paper III, Figure 7, 8). Although higher N concentrations were detected after rooting, plant available N, surprisingly, was reduced (rather than elevated) on rooted compared to non-rooted plots. A possible explanation for this might be that N was removed by plant uptake, immobilized by microorganisms, or lost through leaching and erosion. However, as total plant cover and numbers of sapling were reduced on rooted plots, higher N understory vegetation plant uptake cannot be responsible for the lack of plant available N. Yet, it is possible, that trees took up available N on rooted plots through their roots (as suggested by Singer et al. 1984). We did not assess N uptake by tree roots or tree growth, but this would give valuable and complementary insights and therefore would be worthwhile future research. Further, the higher microbial biomass C found on rooted plots (Paper IV, Figure 3) could have led to an immobilization of N and therefore be responsible for reduced N availability. Finally, the reduced plant cover (and therefore reduced nutrient uptake, paper III, Figure 5) and reduced bulk density on rooted plots (Paper I, Table 3) could have led to accelerated soil erosion and nutrient leaching, which again could have reduced plant available N.

Besides the studies about wild boar rooting effects (Paper III, Table 1), we are aware of only one study that looked at soil disturbance effects of large mammals that we can compare our findings to. Tardiff and Stanford (1998) found positive effects of grizzly bear digging on NH_4^+ -N and NO_3^- -N availability in subalpine meadows of Glacier National Park, Montana,

United States. However, because of differences in site conditions (climate, forest type, soil type, etc.), disturbance intensity, time span between disturbance and sampling, and numerous other factors between the studies, comparisons remain difficult and must be handled with care.

Boar rooting might be comparable to soil disturbances caused by forest management practices – especially soil scarification and contour ripping that mix the surface litter layer with the mineral soil. However, systematic, large-scale forest management practices reveal other disturbance patterns than the randomly distributed rooting patterns caused by wild boars. In addition, almost all forest management practices are performed on clear-cut sites (to improve future growth rates of trees), and these artificially modified ecosystems probably may react differently to soil disturbance than the sites of this study where intact canopy cover was maintained.

Overall Conclusion

The results of this study significantly improve our knowledge on how wild boars may alter forest ecosystems properties, which will be relevant for future discussions about wild boars and their sustainable management. If wild boar populations continue to increase in size and to expand their range in Switzerland, the effects of wild boar rooting on forest ecosystem properties will likely become more pronounced than those that were observed in our study.

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