Impacts of hemiparasitic plants on the vegetation and biogeochemical cycling in two contrasting semi-natural grassland types

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IMPACTS OF HEMIPARASITIC PLANTS ON THE VEGETATION AND BIOGEOCHEMICAL CYCLING IN TWO CONTRASTING SEMI-NATURAL GRASSLAND TYPES

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Land and Forest Management
Dutch translation of the title:
Effecten van halfparasitische planten op de vegetatie en nutriënten-dynamiek in twee contrasterende halfnatuurlijke graslandtypes

Illustration on the front cover:
Heath-grassland vegetation with beautifully flowering
Polygala serpyllifolia at the ‘Hooiput’ nature reserve

Citation:


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“In damp meadows in summer we have the ragged robin (Lychnis flos-cuculi), the spotted orchis (O. maculata), and the yellow rattle (Rhinanthus Crista-galli)”

(Alfred Russel Wallace, 1889)

Dat zei de natuurkundige als voorbeeld dat bloemen die samen bloeien sterk verschillen in kleur en vorm, zodat bestuivende insecten zich eenvoudig aan één soort houden. Nu weten we ook dat *Rhinanthus* zelf het voorkomen van beide andere soorten in de hand werkt. Dit werk kwam tot stand dankzij de medewerking van vele mensen. Hen wil ik graag een woordje van dank toeschrijven.

Voorjaar 2008, bijna afgestudeerd. Ik vroeg aan Kris of er geen mogelijkheden waren om een doctoraat te starten. Liefst iets rond klimaatverandering. Hij vertelde met zo’n enthousiasme over halfparasieten, dat ik het meteen goed vond. Ratelaar kende ik – mijn zus zei er vroeger ‘rammelaar’ tegen als we door de Bourgoyen liepen, heidekartelblad had ik nog niet gezien. Kris, heel erg bedankt dat je me deze unieke kans aanbood. Ik was steeds welkom met mijn vragen, en je wist me telkens weer te motiveren om er voluit voor te gaan. Mijn periode in Gontrode wordt nu nog verlengd. Het wordt weerom een boeiend jaar.

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Hemiparasitic plants from the family *Orobanchaceae* play a key role in the conservation of species-rich semi-natural grasslands. They can alter plant community composition and diversity by both parasitism and litter effects. The drainage of resources by the hemiparasite suppresses preferred host species to the benefit of non-host species. On the other hand, hemiparasitic litter inputs enhance nutrient cycling with indirect positive effects on both host and non-host. When parasitism effects dominate, decreased host and total biomass are expected to result in an increased diversity of the non-host community. In contrast, when litter effects compensate for the biomass loss due to parasitism, minimal to no changes in diversity, minor changes in host biomass and an increase in total biomass are expected. The relative importance of the litter pathway is expected to increase with decreasing nutrient status of the ecosystem.

Here we report on litter and net community effects of two native hemiparasitic plant species growing in vegetation types with a contrasting nutrient status: *Rhinanthus angustifolius* C.C. Gmel. favoring mesotrophic grasslands (3 sites) and *Pedicularis sylvatica* L. growing in oligotrophic heath-grasslands (3 sites). We first linked hemiparasitic litter nutrient returns to the net effect on biomass production; second, we assessed the net effect of both hemiparasites on species abundances and seedling recruitment; third, we studied the impact of hemiparasitic and non-parasitic litter on gross nitrogen (N) transformations in the soil; and forth, we traced N uptake from hemiparasitic litter by the vegetation.

We compared nutrient returns from *Rhinanthus* and *Pedicularis* litter with nutrient stocks in the vegetation and in the soil and assessed nitrogen release rates from both hemiparasitic litter types. The net effect of the hemiparasites on aboveground biomass was studied by a hemiparasite removal experiment. We found that *Pedicularis* litter N, phosphorus (P) and
Summary

Potassium (K) returns to the soil were higher compared to *Rhinanthus* litter and even more so when expressed relative to nutrient amounts in the vegetation. *Pedicularis* litter also released more N to the soil over an eight-month period. *Rhinanthus* had a negative effect on total, graminoid and forb biomass. In contrast, *Pedicularis* did not significantly affect aboveground biomass. These results support that litter effects are more important when nutrients are more limiting.

In the same hemiparasite removal experiment, the effect of both hemiparasites on individual species abundances and seedling establishment after seed addition were studied. *Rhinanthus* had both positive and negative effects on individual species, while *Pedicularis* only had negative effects on some species. The high variation within growth forms suggested that they are not a good predictor of vulnerability to parasitism. Both *Rhinanthus* and *Pedicularis* increased seedling establishment of two of the sown species. Together, these results suggest that the effect on community composition is stronger for *Rhinanthus* compared to *Pedicularis*.

In a laboratory experiment, control soils, soils amended with hemiparasitic litter and soils amended with a non-parasitic litter mixture were incubated. Using $^{15}$N labeling of the ammonium and nitrate pools coupled to a numerical tracing model, we quantified gross N transformation rates. *Rhinanthus* and *Pedicularis* litter additions increased N cycling relative to non-parasitic litter additions. In contrast to expectations based on the higher N release rate from *Pedicularis*, *Rhinanthus* had the strongest effect on gross N transformations in the soil.

After addition of $^{15}$N labeled *Rhinanthus* and *Pedicularis* litter to field plots, we traced $^{15}$N in the vegetation. The overall percentage of vegetation N derived from litter ($N_L$) was not higher than 1%. In agreement with the higher effect of *Rhinanthus* litter on gross N transformation in the soil, the $N_L$ values were higher at *Rhinanthus* sites compared to *Pedicularis* sites. Interestingly, we found that $N_L$ values were positively related with leaf traits typical for a fast-growth strategy.

Our results considerably improved our understanding of litter and net effects of *Rhinanthus* and *Pedicularis* on community composition in semi-natural grasslands. Most strikingly, short-term effects of *Pedicularis* litter on N cycling were smaller even though more N had returned to the soil compared to *Rhinanthus* litter.
Halfparasitische planten van de *Orobanchaceae* familie hebben een sleutelrol in het behoud van soortenrijke halfnatuurlijke graslanden. De invloed die ze uitoefenen op de soortensamenstelling en diversiteit zijn het gevolg van zowel parasitische als strooiseffecten. Het onttrekken van water en nutriënten door de halfparasieten onderdrukt gastheersoorten ten voordele is van niet-gastheersoorten. Anderzijds versnelt het snel afbreekbaar strooisel van halfparasiet de nutriëntencyclus, wat de groei van zowel gastheersoorten als niet-gastheersoorten bevordert. Wanneer de parasitische effecten domineren, verwachten we dat de gastheerbiomassa en de totale biomassazullen afnemen, met als gevolg een stijging in de diversiteit van de niet-gastheersoorten. Echter, als de strooiseffecten de biomassavermindering als gevolg van parasitisme compenseren, verwachten we geen effect op de diversiteit, geringe effecten op de gastheerbiomassa en een stijging in de totale biomass. Ten slotte worden strooiseffecten verwacht aan belang te winnen in ecosystemen met lagere nutriëntenstatus.

We bestudeerden strooiseffecten en het netto effect op de plantengemeenschap voor twee halfparasitische planten uit vegetatietypes met contrasterende nutriëntenstatus: *Rhinanthus angustifolius* C.C. Gmel. (grote ratelaar) in matig voedselrijk grasland (3 locaties) en *Pedicularis sylvatica* L. (heidekartelblad) in heischraal grasland (3 locaties). We onderzochten de hoeveelheid nutriënten in het halfparasitische strooisel en het netto effect van de parasiet op biomassaproductie; het netto effect van beide halfparasieten op de abundantie van andere plantensoorten en op de rekrutering van zaailingen; het effect van halfparasitisch en niet-parasitisch strooisel op stikstof (N) transformaties in de bodem; tenslotte traceerden we N opname uit halfparasitisch strooisel door de vegetatie.

We vergeleken vrijgave van nutriënten uit *Rhinanthus* en *Pedicularis* strooisel met de hoeveelheid nutriënten in de vegetatie en in de bodem. Het netto effect van halfparasieten op de bovengrondse biomassa werd
Samenvatting

bestudeerd in een wiedexperiment. We vonden dat Pedicularis strooisel meer N, fosfor (P) en kalium (K) terug op de bodem bracht in vergelijking met Rhinanthus strooisel. Ook de vrijgave van N uit Pedicularis strooisel was hoger tijdens de eerste 8 maanden. Rhinanthus verminderde de totale en de gras- en kruidachtige biomassa. Pedicularis had geen significant effect op de bovengrondse biomassa. Deze resultaten suggereren dat strooiseleffecten belangrijker zijn wanneer de nutriëntenbeschikbaarheid lager is.

Het effect van beide halfparasieten op soortabundanties en de vestigingskansen van zaailingen werden bestudeerd in hetzelfde wiedexperiment. Rhinanthus had zowel positieve als negatieve effecten op individuele soorten, terwijl Pedicularis alleen negatieve effecten had. Deze resultaten suggereren dat het effect op de soortensamenstelling sterker is voor Rhinanthus in vergelijking met Pedicularis. De hoge variatie binnen groeivormen toonde aan dat niet alle grassen goede gastheren zijn, en niet alle kruidachtigen slechte. Zowel Rhinanthus als Pedicularis verhoogden het aantal zaailingen dat zich kon vestigen van twee ingezaaide soorten.

In een laboratoriumproef incubeerden we bodems met halfparasitisch strooisel, bodems met een niet-parasitische strooiselmengsel en bodems zonder strooisel. De bruto N transformatiesnelheden in de verschillende bodems werden geschat. Hiervoor werden gebruik gemaakt van $^{15}$N aanrijking van de ammonium en nitraat pools en een numeriek tracing model. Rhinanthus en Pedicularis strooisel verhoogden de N dynamiek meer dan niet-parasitisch strooisel. In tegenstelling tot wat we zouden verwachten op basis van de hogere vrijgave van N uit Pedicularis strooisel, had Rhinanthus strooisel een groter effect op de N transformaties in de bodem.

Na toediening van $^{15}$N-gemerkt Rhinanthus en Pedicularis strooisel in het veld, werd $^{15}$N getraceerd in de vegetatie. Het algemeen percentage van de N in de vegetatie dat bekomen werd uit het strooisel ($N_i$) was niet hoger dan 1%. In overeenstemming met het groter effect van Rhinanthus strooisel op N transformaties in de bodem, waren de $N_i$-waarden hoger in de gastheervegetatie van Rhinanthus in vergelijking met deze van Pedicularis. We vonden een positief verband tussen de $N_i$-waarden in soorten en hun bladkarakteristieken die typisch zijn voor een snelle groeistrategie.

Onze resultaten verhoogden het inzicht in strooisel en netto effecten van Rhinanthus en Pedicularis op de samenstelling van plantengemeenschappen in halfnatuurlijke graslanden. Opmerkelijk was dat de korttermijn effecten van Pedicularis strooisel op de N cyclus kleiner waren ondanks dat Pedicularis strooisel meer N vrijgaf dan Rhinanthus strooisel.
List of abbreviations and definitions

Abbreviations

\(^{15}\)N  stable isotope of nitrogen with mass 15
AIC  Akaike information criterion
C  carbon
DOM  dissolved organic matter
DW  dry weight
ECM  ectomycorrhiza
ERM  ericoid mycorrhiza
K  potassium
LNC  leaf nitrogen concentration
LPC  leaf phosphorus concentration
MIT  mineralization-immobilization turnover
N  nitrogen
\(\text{NH}_4^+\)  ammonium
\(\text{NO}_3^-\)  nitrate
\(\text{N}_{\text{org}}\)  organic nitrogen
P  phosphorus
\(P\)  statistical significance
\(R^2\)  coefficient of determination
SD  standard deviation
SE  standard error
SLA  specific leaf area (leaf area / leaf dry weight)
SOM  soil organic matter
\(t\)  test statistic (\(t\)-value)
### Abbreviations and definitions

#### Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>growth form</td>
<td>part of the vegetation such as graminoids (grasses, sedges and rushes), hemiparasites, non-parasitic forbs, ericaceous shrubs and saplings (young trees)</td>
</tr>
<tr>
<td>mesotrophic</td>
<td>moderately high nutrient availability</td>
</tr>
<tr>
<td>oligotrophic</td>
<td>low nutrient availability</td>
</tr>
<tr>
<td>potential host biomass</td>
<td>total aboveground biomass without the hemiparasite</td>
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Earth’s biological diversity is seriously threatened by human-induced changes in biogeochemical cycles, land use and mobility of biota (Chapin et al. 2000). Species diversity determines the functioning of ecosystems because ecosystem processes depend on species traits. Some species, referred to as keystone species (Paine 1969), play a key role in maintaining the organization, functioning and diversity of their ecological community (Mills et al. 1993). This introductory chapter will focus on the importance of temperate, semi-natural grasslands for biodiversity conservation (1.1) and elucidate the key role of parasitic plants in shaping the community structure and diversity (1.2). Towards the end of the introduction, the two hemiparasitic plant species
that are studied in detail are briefly described (1.3) and the general aims and outline of the thesis are given (1.4).

1.1 Semi-natural grasslands are biodiversity hotspots

At a small spatial grain (≤ 50 m²), the highest levels of vascular plant species richness are found in semi-natural, oligo- to mesotrophic, temperate grasslands – mostly managed by periodic mowing (Wilson et al. 2012). In Europe, these vegetation types with high conservation value have mostly evolved under centuries-long traditional agricultural and nature management (Vos and Meekes 1999; Adriaens et al. 2006; Hajkova et al. 2011); see Zwaenepoel et al. (2002c) for details on Flemish grasslands. Due to changing societal demands since the mid-18th century, a vast area of semi-natural grassland was transformed to highly productive species-poor grassland, cropland and built-up land (Butaye et al. 1999; Smit et al. 2008). In addition, management intensification has led to degradation of the remaining grasslands (Vos and Meekes 1999; De Becker 2004), resulting in species loss (Poschlod and WallisDeVries 2002). Key processes driving species loss in semi-natural grasslands are eutrophication (Hautier et al. 2009), primarily caused by fertilizer use, and soil acidification, caused by atmospheric nitrogen (N) and sulfur (S) deposition from agricultural and industrial emissions (Stevens et al. 2004; Stevens et al. 2011); see also the introduction in the PhD thesis of Els Ameloot (Ameloot 2007). The restoration of species-rich semi-natural grasslands has been a top priority in Belgium and neighboring countries since the 1970s (Bakker 1989) and aims at (re-)creating the proper environmental conditions and vegetation structure of the targeted community (Lindborg and Eriksson 2004). In 2010, the Flemish government set its targets for the conservation, expansion and restoration of semi-natural grasslands.
within the framework of the European Natura 2000 network; for example, 257 ha extra species-rich heath-grassland and 900-1650 ha extra oligotrophic lowland meadows are foreseen (Belgisch Staatsblad, 5 November 2010). Restoration efforts aiming at the creation of favorable environmental conditions – such as topsoil removal (e.g., Verhagen et al. 2001), phytoextraction of phosphorus (van der Salm et al. 2009) and rewetting – are often insufficient because of the lack of viable seeds in the seedbank, the limited seed dispersal from remnant populations in fragmented landscapes and the lack of suitable microsites for seedling recruitment (Bakker and Berendse 1999; Kiehl et al. 2010).

1.2 Hemiparasitic plants as keystone species

Parasitic plants – in contrast to autotrophic plants – rely on host plants for (part of) their supply of water, carbon (C) and other nutrients (Kuijt 1969). With about 4100 species in 19 families, they represent 1% of the angiosperms worldwide (Nickrent and Musselman 2004). Parasitic plants are categorized according to the ability to complete their life cycle without a host (obligate and facultative parasites), the place of attachment to the host (stem and root parasites), whether they are photosynthetic (hemiparasites) or acquire carbon exclusively from host plants (holoparasites), and their host specificity (generalists and specialists). In temperate semi-natural grasslands, root hemiparasites of the Orobanchaceae form a well-defined functional group (ter Borg 1985; Press 1998). They can be considered keystone species (Quested et al. 2003b) as they can have profound effects on community structure and plant species diversity (Press and Phoenix 2005). Press (1998) proposed that the net effect of hemiparasites depends on the relative
influence of parasitism and the input of litter by the hemiparasite (Figure 1.1). This was – for the first time – demonstrated for *Rhinanthus minor* (Fisher et al. 2013).

### 1.2.1 The parasitism pathway

The parasitism pathway refers to direct negative effects on host species and indirect positive effects on non-host species. Hemiparasitic plants form haustorial connections to host species through which they take up water, nutrients and carbon compounds, which results in performance reduction of the host species (Kuijt 1969; Pate 1995; Press 1995). Most hemiparasitic plants are generalists, but show high levels of host preference.

![Figure 1.1 Parasitism and litter pathways by which hemiparasites potentially affect community structure and composition.](image)

**Parasitism pathway**: parasitism reduces host biomass. Reductions in host biomass are often greater than increases in hemiparasite growth as hemiparasites often have low nutrient-use efficiencies, which leads to reduced community productivity. The reduction in host plant biomass by the hemiparasite can increase community diversity by allowing the expansion of non-host subordinate species. The establishment of new species can be increased due to suppressed host biomass and ‘gaps’ left in the vegetation after the death of the hemiparasite. **Litter pathway**: high-quality litter from hemiparasites can enhance decomposition, which makes resources more readily available to the plant community. Therefore, the input of hemiparasite litter can increase primary productivity and reduce the effect of parasitism on diversity as competition for nutrients will be reduced. The increased productivity can, in turn, negatively affect the establishment chances of new species. Compiled from Press et al. (1999) and Spasojevic and Suding (2011)
Introduction

(Gibson and Watkinson 1989; Gibson and Watkinson 1991; Musselman and Press 1995; Cameron 2004; Press and Phoenix 2005). Parasitism thus changes the competitive relations between preferred and non-preferred hosts in the vegetation, with possible effects on diversity and composition (Gibson and Watkinson 1991; Matthies 1996; Press et al. 1999). For example, in grasslands infected with *Rhinanthus* spp., the decrease of total, graminoid and legume biomass is thought to alter species composition in favor of non-leguminous forbs and increase local diversity (Ameloot et al. 2005). In the absence of strong host preferences, suppression of the dominant species in the vegetation is more likely because of their higher root densities which increase the chance of encounters between parasite and host roots (Davies et al. 1997). This favors subordinate and newly arrived species and consequently changes community composition and increases species diversity (Gibson and Watkinson 1991; Davies et al. 1997; Ameloot et al. 2005), but see Gibson and Watkinson (1992). Furthermore, parasitic plants often grow at high densities (e.g., up to 350 individuals m\(^{-2}\) for *Rhinanthus angustifolius*, personal observation) and die off early in the growing season, thus creating vegetation ‘gaps’, which can facilitate the establishment of new species (Joshi et al. 2000; Pywell et al. 2004) and consequently increase diversity.

1.2.2 The litter pathway

A second – more indirect – pathway through which hemiparasites can affect vegetation structure and diversity is via their litter input. Hemiparasitic plants act as a sink for water and solutes from the host and accumulate nutrients in their tissue as a result of high transpiration rates (Gauslaa 1990; Gauslaa and Odasz 1990; Ehleringer and Marshall 1995; Pate 1995; Phoenix and Press 2005); therefore, hemiparasites produce good-
quality litter (e.g., low C:N ratio, high calcium content) that mineralizes faster than litter of co-occurring species and consequently increases nutrient inputs to the soil (Seel and Press 1993; Press 1998; Press et al. 1999; Quested et al. 2002; Quested et al. 2003a). Several studies reported positive effects of the presence of hemiparasites or their litter on N cycling. Quested et al. (2003a; 2003b) found a 42% increase of total annual N input to the soil in the vicinity of the hemiparasite *Bartsia alpina* and an increase in plant growth for species grown with *B. alpina* litter compared to litter of co-occurring species. In a mesocosm study, Bardgett et al. (2006) reported higher N mineralization rates in pots with *Rhinanthus minor* compared to pots without the hemiparasite. In a $^{15}$N tracing experiment, Ameloot et al. (2008) observed that $^{15}$NH$_4$$^{15}$NO$_3$ (ammonium nitrate) added to the soil was more diluted in plots parasitized with *Rhinanthus* spp. compared to control plots, which suggests larger soil N pools in parasitized plots. March and Watson (2010) reported that the mistletoe *Amyema miquelii* increased annual litter N returns to the soil with 65% in temperate eucalypt forest, but the effect on phosphorus (P) and potassium (K) returns was even higher. Spasojevic and Suding (2011) found that *Castilleja occidentalis* litter – alone and in mixtures – released N faster than litter from co-occurring species. For model grassland communities in a mesocosm study, Fisher et al. (2013) found that *Rhinanthus minor* litter increased total, grass and legume aboveground biomass.

### 1.2.3 The net effect

The net effect of hemiparasites on the vegetation will depend on the relative importance of the parasitism and litter pathways (see 1.2.1 and 1.2.2 above). When parasitism effects dominate, hemiparasites are expected to decrease host and total
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community productivity and increase diversity of the non-host community (Spasojevic and Suding 2011). For example, *Rhinanthus minor* suppressed host community biomass and increased diversity despite a parasite-driven increase in N mineralization of 105-174% (Bardgett et al. 2006). In line with these findings, Fisher et al. (2013) showed that *R. minor* litter only partially negates grass and total biomass reductions caused by parasitism.

The increase in nutrient availability caused by hemiparasitic litter inputs might also compensate for the biomass loss due to parasitism, resulting in a net biomass increase. In that case, minimal to no changes in diversity, minor changes in host biomass and an increase in total biomass are expected at the community level. For example, *Castilleja occidentalis* was associated with an almost twofold increase in productivity (Spasojevic and Suding 2011). Similar to nutrient addition experiments, which showed an increase in graminoid biomass associated with a loss of mainly small perennial grass and forb species (Silvertown et al. 2006; Hejcman et al. 2007; Hautier et al. 2009; De Schrijver et al. 2011), hemiparasitic litter feedbacks might reduce local species diversity. Yet, Spasojevic and Suding (2011) found only a weak effect of hemiparasitic litter on species abundance and no effect on diversity or species composition.

It is expected that litter effects will be particularly important in arctic or alpine ecosystems, where plant growth is strongly nutrient-limited (Press 1998). In these ecosystems, hemiparasites short-circuit nutrient cycles, not only by producing high-quality litter that decomposes fast, but also by unlocking nutrients from slow-growing, long-lived perennials. Longer-lived hemiparasites, such as *Castilleja occidentalis* and *Bartsia alpina*, may also compensate for the negative community-level effect of
parasitism due to the concentration of nutrients in patches from a relatively large area (Quested et al. 2003b; Spasojevic and Suding 2011).

### 1.2.4 Unresolved issues

While many studies reported on the net effect of hemiparasitic grassland plants on aboveground biomass, and a growing number of studies have looked at the litter pathway, only a few studies combined both (e.g., Bardgett et al. 2006; Spasojevic and Suding 2011; Fisher et al. 2013). Moreover, no studies included different hemiparasitic species from contrasting vegetation types. In this thesis we studied both litter and net effects in one integrated study on two hemiparasitic plant species from semi-natural grassland types with a contrasting nutrient status. Litter effects on nutrient cycling were focused on N as it is an easy to trace nutrient ($^{15}\text{N}$). We expected the litter pathway to be more important in the grasslands where nutrient availability is low, while we expected the net effect to be more important in the grasslands with higher nutrient availability.

### 1.3 Study species and communities

Here we focused on semi-natural grasslands in Flanders (northern Belgium). Out of the native hemiparasitic plant species (e.g., *Euphrasia stricta, Odontites verna, Pedicularis palustris, Rhinanthus minor*), we selected two short-lived hemiparasites that occur in plant communities on opposite sides of a natural gradient in plant-available nutrients: *Rhinanthus angustifolius* C.C. Gmel. typically growing in mesotrophic grasslands belonging to the *Molinio-Arrhenatheretea* class (*sensu* Zuidhoff et al. 1996) (see also Zwaenepoel et al. 2002a), and *Pedicularis sylvatica* L. thriving in relatively oligotrophic heath-grasslands belonging to the *Nardetea* class (*sensu* Swertz et al. 1996) (see also
Zwaenepoel et al. 2002b) (Figure 1.2). Hereafter they are referred to as *Rhinanthus* and *Pedicularis*. Belgium is located well within both species’ native ranges, which overlap in most of Northwestern continental Europe (Figure 1.3).

Like most *Orobanchaceae* species, the annual *Rhinanthus* and the biennial *Pedicularis* are short-lived, have no capacity for clonal growth and have rather low seed production and low seed bank longevity compared to non-hemiparasitic plants (Bekker and Kwak 2005). This combination of traits makes them particularly vulnerable to extinction as a result of habitat deterioration and fragmentation. Both species have declined during the last decades and their present occurrence within Flandern is largely restricted to nature reserves (Van Landuyt et al. 2006). While *Rhinanthus angustifolius* occurs locally in the whole of Flandern, *Pedicularis sylvatica* is rare in the Campine region and very rare in the rest of Flandern (Figure 1.4).
Figure 1.3 Distribution of Rhinanthus angustifolius (top) and Pedicularis sylvatica (bottom) in Europe. The species are common within the dashed area and also occur outside this area where dots (exact indication) or open circles (approximate indication) are drawn. From: Hultén and Fries (1986)
Introduction

1.4 Aims and outline of the thesis

This thesis aims at better understanding the net effects of hemiparasites on the vegetation structure in semi-natural grasslands (chapter 2 and 3), and, in particular, at achieving a better insight in the importance of the litter pathway and its potential consequences for species composition (chapter 2, 4 and 5) (Figure 1.5). Throughout all chapters, *Rhinanthus* and *Pedicularis* are used as study species and differences between them are discussed. In chapter 2 we evaluate the effects of *Rhinanthus* and *Pedicularis* removal on the aboveground biomass of individual growth forms and the total vegetation. To estimate the importance of the litter pathway, we determined the production and nutrient content of hemiparasitic litter as well as the nutrient pools in the soil and those removed by mowing. Studies on the effects of hemiparasitic plants on
Chapter 1

vegetation structure, including chapter 2, have generally grouped species into growth forms, whereas pot experiments have been carried out to study effects on individual species. In chapter 3, the net effects of *Rhinanthus* and *Pedicularis* weeding on the vegetation are studied *in situ* and at the species level. We looked at effects on the abundance of species in the vegetation as well as on seedling establishment of a selected number of species.

In chapter 4 – the only laboratory experiment in this thesis – the effects of *Rhinanthus* and *Pedicularis* litter on gross N transformation rates in the soil is studied using a state-of-the-art modeling approach based on $^{15}$N pool dilution and tracing techniques. Soils amended with hemiparasitic litter are compared to soils amended with a litter mix of co-occurring species and control soils without litter. Does hemiparasitic litter increase the turnover of N pools in the soil and, as a consequence, the availability of N to plants?

Next, in chapter 5, $^{15}$N-labeled *Rhinanthus* and *Pedicularis* litter is applied to field plots and $^{15}$N is traced in the shoots of co-occurring species. The amount of N each species derived from the added litter is then related to plant traits that determine the species’ N economy and growth strategy. Are hemiparasites promoting fast-growing species that are more adapted to highly fertile environments?

In the final chapter, the results of chapters 2 to 5 are summarized and more general conclusions are made (chapter 6). What did we learn about the net effects of hemiparasites on the vegetation – especially on species composition, what is the importance of the litter pathway and what are its consequences? Are the species favored by the litter pathway the same as the ones benefiting from the parasitism pathway? In addition, several suggestions for further research are proposed.
**Figure 1.5** Schematic outline of the four main chapters (dashed boxes) in this thesis: chapter 2 studies the effect of hemiparasite weeding on the aboveground biomass of growth forms and discusses the potential contribution of the litter pathway by quantification of hemiparasitic litter amounts and their decomposition dynamics; chapter 3 elaborates on net vegetation effects at the species level; chapter 4 evaluates the effect of hemiparasitic litter and a litter mix of co-occurring species on N transformation rates in the soil; and finally, chapter 5 traces 15N in the shoots of co-occurring species after addition of 15N-labeled litter. Chapters 2, 3 and 5 are carried out in the field (light-grey zone); chapter 4 is a lab experiment (white zone). Chapters 4 and 5 make use of 15N stable isotope methods.
Effects of *Rhinanthus* and *Pedicularis* on biomass production and litter nutrient returns


**Abstract**

Hemiparasitic can substantially change plant community structure; the drainage of host resources has a direct negative effect on host biomass and, as a consequence, promotes non-host biomass production (parasitism pathway); on the other hand, hemiparasitic litter inputs can enhance nutrient cycling which may have an indirect positive effect on both host and non-host biomass production (litter pathway). We evaluated the net effect of both pathways on total shoot biomass (with and without the hemiparasite) and shoot biomass of graminoids, forbs and ericaceous shrubs using a removal experiment in three sites infested with the annual *Rhinanthus angustifolius*, and three sites infested with the biennial *Pedicularis sylvatica*. We addressed the potential importance of litter effects by determination of litter quantity and quality, as well as modeling nitrogen (N) release during decomposition. In the second year after removing the hemiparasites, total plant biomass at *Rhinanthus* sites was 24% higher in weeded plots than in control plots,
while weeding had no significant effect at *Pedicularis* sites. The increase in total biomass following *Rhinanthus* removal was mainly due to a higher biomass of graminoids. The amount of hemiparasite litter produced by *Rhinanthus* was only half of that produced by *Pedicularis*, both with similar N content. The N amount in the litter was 9% and 30% of the N amount removed by mowing for *Rhinanthus* and *Pedicularis* sites. Within two months, about 45% of the N in both hemiparasitic litter types was released by decomposition. Our results suggest that in addition to the suppression of host biomass due to parasitism, also positive litter feedbacks on host and non-host biomass – via an increase in nutrient availability – affect plant community structure. We propose that, depending on the particular hemiparasite and/or site conditions, these positive litter feedbacks on shoot biomass can compensate the negative effect of parasitism.

### 2.1 Introduction

Hemiparasitic plants can be considered keystone species (Quested et al. 2003b) as they may have profound effects on community structure and plant diversity (Press and Phoenix 2005). Press (1998) proposed that the net effect of hemiparasites is dependent on the relative influence of parasitism and litter effects (1.2.1 and 1.2.2 in chapter 1).

It has been suggested that the increased nutrient availability in the soil due to the presence of hemiparasites (the litter pathway) might counteract the biomass decrease due to parasitism to some degree (the parasitism pathway) (Quested et al. 2003b; Ameloot et al. 2008). More generally, Spasojevic and Suding (2011) proposed that, when effects of parasitism dominate, hemiparasites should increase diversity of the non-host community and decrease host and total community productivity. In contrast, if litter effects on nutrient supply compensate for the reduction in host biomass, minimal to no changes in diversity as well as minor changes in host biomass and an increase in total biomass are expected at the community level. Few studies reported on the potential
Biomass and litter decomposition

importance of both parasitism and litter effects. For example, in a mesocosm study of Bardgett et al. (2006), *Rhinanthus minor* suppressed host community biomass and increased diversity, despite a parasite-driven increase in N mineralization by 105-174%. In contrast, in an observational study, Spasojevic and Suding (2011) found *Castilleja occidentalis* to be associated with an almost twofold increase in productivity and related this to the higher N-release from litter mixtures including *Castilleja* compared to without. In contrast to short-lived *Rhinanthus*, it has been suggested that longer-lived hemiparasites in nutrient-poor systems, such as *Castilleja occidentalis* and *Bartsia alpina* may compensate for the negative community-level effect of parasitism due to the concentration of nutrients in patches from a relatively large area (Quested et al. 2003b; Spasojevic and Suding 2011). Therefore, in the study of Spasojevic and Suding (2011), litter effects might dominate over the effects of parasitism only locally – rather than at the community-level.

In this chapter, we evaluate the net community-level effect (resulting from parasitism and litter pathways) *in situ* for two short-lived hemiparasitic plants from different genera (*Rhinanthus* and *Pedicularis*) keeping the mowing regime – vital to the presence of the hemiparasite in the study sites – unchanged. We expect an increase of aboveground biomass after hemiparasite removal; in addition, we expect this increase to be most pronounced in the graminoid component. Subsequently, we discuss these net effects on aboveground biomass with respect to the potential contribution of the litter pathway using data on litter amounts, its chemical composition and its decomposition dynamics.
2.2 Materials and methods

2.2.1 Study systems

Two grassland hemiparasitic plant species differing in life history traits and growing in contrasting environments were selected for this study: (1) the annual *Rhinanthus angustifolius* C.C. Gmel., typically growing in mesotrophic grasslands belonging to the *Molinio-Arrhenatheretea* class (*sensu* Zuidhoff et al. 1996), and (2) the biennial *Pedicularis sylvatica* L., thriving in relatively oligotrophic heath-grasslands belonging to the *Nardetea* class (*sensu* Swertz et al. 1996). Within the study area, *Rhinanthus* (*Orobanchaceae*) is flowering in June, turning fields yellow. Shortly after flowering, most leaves are shed leaving only brown stems and seed capsules in July, when the grasslands are usually mown. *Pedicularis* (*Orobanchaceae*) seedlings start to grow in May and produce only leaves the first year which die off in winter. The second year in March, the plant re-sprouts from the taproot and produces several prostrate branches. *Pedicularis* has conspicuous purple flowers late in May and when seeds are ripe in July, plants die off. Mowing occurs between August and early October, but only the *Pedicularis* tips of upright branches are removed (about 20% of the shoots, personal observation). Because *Pedicularis* plants are distributed in patches, we restricted the studied community to these patches. For both hemiparasites, three sites were chosen in nature reserves in the east of Flanders, which is well within both species’ native ranges. For *Rhinanthus*, these sites are: Doode Bemde (Rhin-D), Achter Schoonhoven (Rhin-A), and Papendel (Rhin-P) (Figure 2.1); and for *Pedicularis*: Walenbos (Pedi-W), Hooiput (Pedi-H), and Langdonken (Pedi-L) (Figure 2.2) (Table 2.1, Figure 1.4 in chapter 1). Soil pH-H2O values range from 5.0 to 5.2 for *Rhinanthus* sites and from 4.5 to 4.6 for *Pedicularis* sites, while soil texture varies greatly between sites. The long-term (1961-1990) mean annual precipitation in
Table 2.1 Overview and comparison of the six experimental sites. Mesotrophic *Rhinanthus* sites (Rhin- ) are D: Doode Bemde, A: Achter Schoonhoven, and P: Papendel; oligotrophic *Pedicularis* sites (Pedi- ) are W: Walenbos, H: Hooiput, and L: Langdonken. Quantitative data (mean ± SE) are from 2009 (Pedi-H from 2010), n = 3 for pH and soil density, n = 6 for species number, hemiparasite cover and weeded hemiparasite biomass and numbers. *Pedicularis* cover is shown separately for second year adults\(^{(a)}\) and seedlings\(^{(s)}\).

<table>
<thead>
<tr>
<th></th>
<th>Mesotrophic <em>Rhinanthus</em> sites</th>
<th>Oligotrophic <em>Pedicularis</em> sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhin-A</td>
<td>Rhin-D</td>
</tr>
<tr>
<td>Latitude</td>
<td>50°58'58&quot;N</td>
<td>50°48'57&quot;N</td>
</tr>
<tr>
<td>Longitude</td>
<td>4°51'39&quot;E</td>
<td>4°38'54&quot;E</td>
</tr>
<tr>
<td>Altitude (masl)</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td><strong>Species richness (0.25 m(^2))</strong></td>
<td>14.3 ± 0.8</td>
<td>21.1 ± 1.0</td>
</tr>
<tr>
<td><strong>Soil properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRB class</td>
<td>Eutric fluvisol</td>
<td>Eutric fluvisol</td>
</tr>
<tr>
<td>USDA texture</td>
<td>Silt loam</td>
<td>Silt</td>
</tr>
<tr>
<td>Drainage class</td>
<td>Moderately poor</td>
<td>(Moderately) poor</td>
</tr>
<tr>
<td>pH-H(_2)O</td>
<td>5.05 ± 0.07</td>
<td>5.18 ± 0.20</td>
</tr>
<tr>
<td>Bulk soil density (g cm(^{-3}))</td>
<td>0.60 ± 0.02</td>
<td>0.52 ± 0.11</td>
</tr>
<tr>
<td><strong>Management</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(^{st}) mowing</td>
<td>Early Aug</td>
<td>Mid Jul</td>
</tr>
<tr>
<td>2(^{nd}) mowing</td>
<td>Sep</td>
<td>Sep</td>
</tr>
<tr>
<td><strong>Hemiparasite properties</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover (%)</td>
<td>53 ± 4</td>
<td>61 ± 4</td>
</tr>
<tr>
<td>Seedling emergence</td>
<td>Apr</td>
<td>Apr</td>
</tr>
<tr>
<td>2(^{nd}) year emergence</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flowering</td>
<td>Early Jun</td>
<td>Late Jun</td>
</tr>
<tr>
<td>Initial weeding (g m(^{-2}))</td>
<td>52 ± 6</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>Initial weeding (n m(^{-2}))</td>
<td>388 ± 48</td>
<td>692 ± 88</td>
</tr>
<tr>
<td><strong>Harvest dates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>1-Aug</td>
<td>16-Jul</td>
</tr>
<tr>
<td>2010</td>
<td>3-Aug</td>
<td>15-Jul</td>
</tr>
</tbody>
</table>
Figure 2.1 Selected *Rhinanthus* sites are situated in the nature reserves ‘Achter Schoonhoven’ in Aarschot (a), ‘Doode Bemde’ in Sint-Joris-Weert (b) and ‘Papendel’ in Begijnendijk (c)
Figure 2.2 Selected *Pedicularis* sites are situated in the nature reserves ‘Hooiput’ in Arendonk (a), ‘Landonken’ in Herstelt (b) and ‘Walensbos’ in Tielt-Winge (c)
this region is 800 mm, and the mean annual temperature is 9.5°C (Royal Meteorological Institute of Belgium). *Rhinanthus* sites have a relatively high local species richness (on average 18 ± 1 species per 0.25 m²) and are characterized by a herbaceous vegetation of perennial graminoids (*Agrostis capillaris, Anthoxanthum odoratum, Juncus acutiflorus, Festuca rubra, Luzula multiflora, Scirpus sylvatica*), legumes (*Lotus uliginosus, Trifolium pratense, Lathyrus pratensis*) and many non-leguminous forbs (e.g., *Centaurea jacea, Prunella vulgaris, Succisa pratensis, Ajuga reptans, Ranunculus acris, Leucanthemum vulgare, Plantago lanceolata, Dactylorhiza maculata, Lychnis flos-cuculi*). *Pedicularis* sites consist of fewer species (on average 12 ± 1 per 0.25 m²) and contain a greater share of (dwarf) shrubs such as *Calluna vulgaris, Erica tetralix* and *Salix aurita*; grasses include *Agrostis canina, Carex pilulifera, Danthonia decumbens, Juncus acutiflorus, Luzula multiflora* and *Molinia caerulea*. The most abundant forb species are *Polygala serpyllifolia* and *Potentilla erecta*.

### 2.2.2 Experimental setup and data collection

**Hemiparasite removal: plant community biomass response**

On all sites, three blocks of approximately 2 x 2 m² were randomly selected within a 20 x 20 m² area in April 2009, i.e., when most *Rhinanthus* and second year *Pedicularis* individuals had emerged. Within each block, four plots of 0.5 x 0.5 m² were randomly selected out of all potential plots that had a minimum of 20% hemiparasite cover and a species composition similar to that of the block. Between 26-May-2009 and 2-Jun-2009 both hemiparasites were weeded in two randomly assigned plots in each block, leaving the other two as untreated controls. The number of plants removed was on average 444
± 38 m$^{-2}$ for *Rhinanthus* and 184 ± 13 m$^{-2}$ for *Pedicularis* (Table 2.1). Thereafter, weeding was repeated monthly until October 2009 and from April to October 2010.

In 2009 and 2010 aboveground biomass of plants in all plots was clipped 2 cm above soil surface between mid-July and early September (Table 2.1), sorted into different growth forms (graminoids, ericaceous shrubs, saplings, hemiparasites, and other forbs) and subsequently oven-dried (48 h at 70°C) and weighed per growth form. In our analysis we only discuss effects on total, graminoid and forb (*Rhinanthus* sites) or ericaceous (*Pedicularis* sites) biomass, since too few data were available for other groups. For Ped-H, no data on shoot biomass were available for 2009 due to miscommunication with the reserve managers. To get some background data on belowground biomass and nutrient stocks at each site (see paragraph on chemical analysis below), a mineral topsoil sample (0-5 cm, diam = 5 cm) was taken next to all plots at the start of the experiment. Samples were pooled per block, dried (96 h at 40°C) and sieved through a mesh of 2 mm to sort out roots.

**Hemiparasitic litter inputs: quality and N release**

Litter of *Rhinanthus* and *Pedicularis* was collected from all sites at the end of June 2009. We randomly gathered an equal number of plants from the three blocks selected for the weeding experiment. We defined ‘litter’ as the part of the shoot involved in nutrient recycling under the applied management regime, i.e., aboveground biomass that would not be removed by mowing. As *Rhinanthus* leaves fall off early in the season, only stems are removed by mowing. Therefore we collected yellowing leaves that came loose easily. *Pedicularis* plants, on the other hand, are low in stature with a rosette of mostly prostrate branches of which only the tips are removed by mowing. We collected total
aboveground plants when they started to turn brown and assumed that 20% of the plant is removed by mowing (removal factor). For each location, eight 20 x 20 cm² litter bags with a mesh size of 1.5 mm were filled with 1.5 or 3 g air-dry (25°C) litter of *Rhinanthus* or *Pedicularis*, respectively, and put in close contact to the ground. The fine mesh size was chosen to prevent loss of undecomposed litter. After three weeks and two, four and eight months, two litter bags were collected at each location. The litter bags were emptied carefully and plants that had grown through the mesh (mostly mosses and grasses) were removed. Samples were dried first at 25°C and weighed to determine mass loss, then dried further (48h at 70°C) and analyzed for C and N concentrations. Initial litter (t=0) was additionally analyzed for lignin concentration.

### 2.2.3 Chemical analysis

All plant samples were dried at 70°C for 48 h and ground with an ultra centrifugal mill (ZM200, Retsch, Germany). Soil samples were dried at 40°C for 96 h after which they were ground with a planetary ball mill (PM400, Retsch, Germany). Subsamples of plant and soil samples were analyzed for total C and N concentration using an elemental analyzer (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (SerCon, UK). Other plant subsamples were digested with HNO₃ (65%) and HClO₄ (70%) in a 5:1 ratio, and soil subsamples were digested with HClO₄ (70%), HNO₃ (65%) and H₂SO₄ (98%) in a 24:5:1 ratio. K⁺ concentrations were measured using flame absorption spectrophotometry, and P was determined colorimetrically by the molybdate method (Scheel 1936). The quality of the chemical analyses was checked by including method blanks, repeated measurements of certified reference samples (CRM 100), and inter-
laboratory tests. Lignin was determined by the Institute for Agricultural and Fisheries Research (ILVO) using a method derived from Van Soest et al. (1991).

2.2.4 Data analysis

We applied mixed effects models using the `lme4` package in R 2.12.1 (the R Development Core Team 2011). We analyze the effect of weeding for 2009 and 2010 data separately using the model $Biomass \sim weeding + (1|location/block)$ in which $Biomass$ is the shoot biomass of either total vegetation, potential host species or one of the growth forms, $weeding$ the independent fixed factor with levels ‘weeded’ and ‘control’, and including random intercepts for $block$ nested within $location$. The nested random effects were added because biomass measurements within the same location or within the same block at a given location are non-independent replicates. We also analyzed the interaction effect between weeding and year ($weeding:year$) using the model $Biomass \sim weeding + year + weeding:year + (year-1|location/block)+(1|plot)$ in which $year$ is a fixed factor with levels ‘2009’ and ‘2010’, and including random intercepts for $plot$ (biomass measurements within the same plot in different years are non-independent) as well as random $year$ effects for block nested within location (the effect of $year$ depends on $location$ and $block$). More details and R code for the selection of the random structure and testing of the fixed effects can be found in appendix A. Models were validated by graphical inspection for normality and homogeneity. A significant weeding:year interaction means that the magnitude and/or direction of the biomass change following weeding has changed between 2009 and 2010. This change (%) was calculated as:
Chapter 2

\[
\left(\frac{W_{2010} - C_{2010}}{C_{2010}}\right) - \left(\frac{W_{2009} - C_{2009}}{C_{2009}}\right) \times 100
\]  

(2.1)

in which \( W_X \) is the aboveground biomass of a weeded plot in year X and \( C_X \) the aboveground biomass of a control plot in year X.

Decomposition data often fit well to a double-exponential equation, where litter is split in a more labile and a more recalcitrant fraction (e.g., Lousier and Parkinson 1976). However, as Rovira and Rovira (2010) argue, it is often not possible to link those fractions with quantifiable organic pools. To fit our data we therefore used their composite-exponential model:

\[
\frac{X_t}{X_0} = e^{-\frac{a - b}{m} t^{m - 1}}
\]  

(2.2)

where \( t \) is the time in days, \( X_0 \) and \( X_t \) are the initial amount of litter and the remaining amount of litter at time \( t \), respectively, and \( a \), \( b \) and \( m \) are model parameters. The model represents Olson’s exponential equation \( \frac{X_t}{X_0} = e^{-kt} \), Olson 1963), in which the decay rate \( k \) in itself decreases exponentially in time from an initial value \( a + b \) (\( t \to 0 \)) to a final value \( a \) (\( t \to \infty \)) with a decay rate \( m \). The main advantage of this model, in contrast to the double exponential model, is that there are no \textit{a priori} assumptions about the internal structure of the decomposing substrate (Rovira and Rovira 2010).

For \textit{Rhinanthus} litter data \( m \) and \( b \) were perfectly correlated (\( r = 1.00 \)), which resulted in high uncertainties about the parameter estimates. Therefore, \( m \) was initially given the same value as estimated for \textit{Pedicularis} litter (0.066). Then we selected a value for \( m \) (0.087) that was used for both litter types assuring that it was within the 95% confidence bounds estimated for \textit{Pedicularis} litter, and while maximizing the coefficient of
determination \( R^2 = 1 - \frac{\text{Residual Sum of Squares}}{\text{Corrected Sum of Squares}} \) for Rhinanthus litter.

SPSS 15.0 for Windows was used to fit this model for the litter bag datasets of Rhinanthus and Pedicularis. The iterative Marquardt-Levenberg algorithm was used to estimate the optimal parameter values.

2.3 Results

2.3.1 Biomass and nutrient stocks

There was little variation between Rhinanthus sites in average shoot biomass (357-368 g m\(^{-2}\)) and carbon (C) content (Table 2.2). Average N content (4.23-4.88 g m\(^{-2}\)) per site varied with biomass, while phosphorus (P) and potassium (K) did not. Rhinanthus itself made up 8-10% of total shoot N (data not shown). Average shoot biomass at Pedicularis sites was lower (184-188 g m\(^{-2}\)), with little variation between the sites for biomass as well as C content. Also the variation in N (2.46-2.79 g m\(^{-2}\)), P and K content was small. Pedicularis made up 9-12% of total shoot N (data not shown).

Total C and nutrient contents in the upper soil layer (0-5 cm) were 1-2 orders of magnitude higher compared to shoot contents in both study systems. Total C (and thus organic matter) was higher and more heterogeneous in Pedicularis sites, compared to Rhinanthus sites. In addition, average C:N:P ratios were lower at Rhinanthus sites (105:9:1) compared to Pedicularis sites (421:18:1). Interestingly, N:P ratios in the shoot biomass reflected those in the soil in both study systems.
Table 2.2 Mean (±SE) for dry weight (DW), C, N, P and K stocks (g m⁻²) in shoot biomass and in the topsoil for six control plots in both grassland types in 2009. Soil biomass was taken from 0-5 cm depth; n = 6 for shoot data, n = 3 for soil data.

<table>
<thead>
<tr>
<th>Shoots</th>
<th>Rhin-A</th>
<th>Rhin-D</th>
<th>Rhin-P</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>368 ± 22</td>
<td>357 ± 12</td>
<td>366 ± 40</td>
<td>364 ± 15</td>
</tr>
<tr>
<td>C</td>
<td>161 ± 9</td>
<td>151 ± 5</td>
<td>159 ± 17</td>
<td>157 ± 6</td>
</tr>
<tr>
<td>N</td>
<td>4.88 ± 0.33</td>
<td>4.23 ± 0.15</td>
<td>4.42 ± 0.39</td>
<td>4.51 ± 0.18</td>
</tr>
<tr>
<td>P</td>
<td>0.38 ± 0.02</td>
<td>0.65 ± 0.04</td>
<td>0.51 ± 0.04</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>K</td>
<td>2.85 ± 0.23</td>
<td>2.75 ± 0.18</td>
<td>4.2 ± 0.54</td>
<td>3.27 ± 0.25</td>
</tr>
<tr>
<td>Soil (0-5 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2496 ± 71</td>
<td>1987 ± 116</td>
<td>1708 ± 6</td>
<td>2063 ± 122</td>
</tr>
<tr>
<td>N</td>
<td>195 ± 12</td>
<td>177 ± 12</td>
<td>144 ± 3</td>
<td>172 ± 9</td>
</tr>
<tr>
<td>P</td>
<td>17.2 ± 0.7</td>
<td>20.9 ± 3.3</td>
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<td>54.6 ± 9.1</td>
<td>101.1 ± 24.2</td>
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</tr>
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</table>

<table>
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<th>Pedi-W</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW</td>
<td>184 ± 24</td>
<td>187 ± 25</td>
<td>188 ± 21</td>
<td>186 ± 13</td>
</tr>
<tr>
<td>C</td>
<td>84 ± 12</td>
<td>85 ± 12</td>
<td>81 ± 9</td>
<td>83 ± 6</td>
</tr>
<tr>
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<td>2.46 ± 0.31</td>
<td>2.79 ± 0.30</td>
<td>2.64 ± 0.16</td>
</tr>
<tr>
<td>P</td>
<td>0.15 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>K</td>
<td>1.66 ± 0.17</td>
<td>1.55 ± 0.21</td>
<td>2.16 ± 0.28</td>
<td>1.79 ± 0.14</td>
</tr>
<tr>
<td>Soil (0-5 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3530 ± 903</td>
<td>2969 ± 891</td>
<td>4740 ± 1085</td>
<td>3746 ± 548</td>
</tr>
<tr>
<td>N</td>
<td>147 ± 17</td>
<td>123 ± 48</td>
<td>202 ± 62</td>
<td>157 ± 26</td>
</tr>
<tr>
<td>P</td>
<td>7.3 ± 0.3</td>
<td>7.6 ± 0.4</td>
<td>11.9 ± 0.9</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>K</td>
<td>34.5 ± 3.0</td>
<td>44 ± 3.2</td>
<td>214.9 ± 35.4</td>
<td>97.8 ± 31.1</td>
</tr>
</tbody>
</table>
2.3.2 Effect of hemiparasite weeding on biomass

In summer 2009, 1.5-3 months after the weeding was initiated (depending on site; Table 2.1), we did not find significant effects on the aboveground biomass for the *Rhinanthus* or *Pedicularis* sites (Figure 2.3). In 2010, however, total biomass (including the hemiparasite) was significantly higher in weeded plots compared to unweeded controls for *Rhinanthus* (+24%, $L = 14.96$, $P < 0.001$), but not for *Pedicularis* (+16%, $L = 2.89$, $P = 0.089$), and potential host biomass (total minus the hemiparasite) was significantly higher compared to controls for both *Rhinanthus* (+41%, $L = 27.20$, $P < 0.001$) and *Pedicularis* (+28%, $L = 4.73$, $P = 0.030$) (Figure 2.3). Considering the biomass data per growth form in 2010, for *Rhinanthus* the graminoid biomass (+47%, $L = 19.19$, $P < 0.001$) and the forb biomass (+20%, $L = 5.71$, $P = 0.017$) was significantly higher in weeded plots compared to the controls, while for *Pedicularis* there was no significant effect on the graminoid biomass (+19%, $L= 2.00$, $P = 0.158$) nor on the ericaceous biomass (+20%, $L = 0.02$, $P = 0.889$).

For *Rhinanthus* sites, the effect of hemiparasite removal on total, potential host and graminoid aboveground biomass increased significantly between 2009 and 2010, indicated by a positive change in relative effect size in combination with a significant weeding:year interaction ($P < 0.001$, Table 2.3); this was not the case for forb biomass. For *Pedicularis* sites, the weeding effect on aboveground biomass did not change significantly ($P = 0.43$ for total biomass, Table 2.3) between the two years for any biomass category.
2.3.3 Hemiparasite litter quantity and decomposition

The amount of *Pedicularis* plant litter was per m² almost double the amount of *Rhinanthus* leaf litter (Table 2.4). Nutrients retained in *Rhinanthus* litter were 9, 9 and 10% of those removed by mowing for N, P and K, respectively. *Pedicularis* litter contained 30, 56 and 72% of N, P and K removed by mowing. Within 8 months, virtually all litter of *Rhinanthus* was decomposed, while about 40% of *Pedicularis* litter still
remained (Figure 2.4a). Both hemiparasites had similar C:N ratios, while the average lignin:N ratio of *Pedicularis* litter (6.75) was twofold higher than that of *Rhinanthus* litter (3.22).

**Table 2.3** The change in relative weeding effect on aboveground biomass between 2009 and 2010 (Equation 2.1). Positive values denote a stronger weeding effect in 2010 compared to 2009. Significance levels of the year:weeding interaction are added: (*) 0.1 > P ≥ 0.05; * 0.05 > P ≥ 0.01; ** 0.01 > P ≥ 0.001; *** P < 0.001. NA: no data available or negligible biomass (see Figure 2.5). See Table 2.1 for site codes.

<table>
<thead>
<tr>
<th></th>
<th>Graminoid</th>
<th>Forb</th>
<th>Ericaceous</th>
<th>Total biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhinanthus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhin-A</td>
<td>69% (**</td>
<td>17%</td>
<td>NA</td>
<td>51% ***</td>
</tr>
<tr>
<td>Rhin-D</td>
<td>20% (*)</td>
<td>-3%</td>
<td>NA</td>
<td>16% (*)</td>
</tr>
<tr>
<td>Rhin-P</td>
<td>37% (*</td>
<td>8%</td>
<td>NA</td>
<td>20% *</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>36% (**</td>
<td>6%</td>
<td>NA</td>
<td>24% ***</td>
</tr>
<tr>
<td><strong>Pedicularis sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedi-H</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pedi-L</td>
<td>38% (*)</td>
<td>NA</td>
<td>10%</td>
<td>28% (*)</td>
</tr>
<tr>
<td>Pedi-W</td>
<td>2%</td>
<td>NA</td>
<td>98%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>18%</td>
<td>NA</td>
<td>21%</td>
<td>17%</td>
</tr>
</tbody>
</table>

The N amount released from the decomposing hemiparasite litter after 2 months was similar for *Rhinanthus* (44%) and *Pedicularis* (46%) litter, while after 8 months the relative N release increased to 94% for *Rhinanthus* and 59% for *Pedicularis* (Figure 2.4b). The absolute N release during decomposition remained highest for *Pedicularis* litter over the whole 8-month period (Figure 2.4c). According to the parameter estimates of the composite-exponential model (Equation 2.2; ), both the initial (a+b) and final (a) mass decay rates were significantly higher for *Rhinanthus* litter than for *Pedicularis* litter. While the final N release rate was significantly higher for *Rhinanthus* litter as well, the initial N release rate was about six times higher for *Pedicularis* litter than for *Rhinanthus* litter.
Table 2.4  Hemiparasitic litter mass, nutrient content and C:N as well as lignin:N ratios for both vegetation types. Values are averages of three 0.5x0.5 m² plots per site, one in each block. See Table 2.1 for site codes

<table>
<thead>
<tr>
<th></th>
<th>Litter mass</th>
<th>Litter nutrients¹ (g m⁻²)</th>
<th>C:N</th>
<th>Lignin:N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g m⁻²)</td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>Rhinanthus sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhin-A</td>
<td>32.0</td>
<td>0.486 (10)</td>
<td>0.045 (12)</td>
<td>0.334 (12)</td>
</tr>
<tr>
<td>Rhin-D</td>
<td>24.2</td>
<td>0.424 (10)</td>
<td>0.053 (8)</td>
<td>0.364 (12)</td>
</tr>
<tr>
<td>Rhin-P</td>
<td>20.0</td>
<td>0.282 (6)</td>
<td>0.043 (8)</td>
<td>0.296 (12)</td>
</tr>
<tr>
<td>Average</td>
<td>25.4</td>
<td>0.396 (9)</td>
<td>0.047 (9)</td>
<td>0.331 (10)</td>
</tr>
<tr>
<td>Pedicularis sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pedi-H</td>
<td>52.7</td>
<td>0.938 (35)</td>
<td>0.119 (80)</td>
<td>1.083 (65)</td>
</tr>
<tr>
<td>Pedi-L</td>
<td>41.9</td>
<td>0.667 (27)</td>
<td>0.074 (44)</td>
<td>1.171 (76)</td>
</tr>
<tr>
<td>Pedi-W</td>
<td>46.3</td>
<td>0.787 (28)</td>
<td>0.095 (53)</td>
<td>1.616 (75)</td>
</tr>
<tr>
<td>Average</td>
<td>47.0</td>
<td>0.794 (30)</td>
<td>0.096 (56)</td>
<td>1.290 (72)</td>
</tr>
</tbody>
</table>

¹ The ratio of litter nutrients over total shoot nutrients (%) are added between parentheses

Table 2.5 Parameter estimates (±SE) and coefficient of determination ($R^2$) for the composite exponential model (Equation 2.1, with $m$ fixed based on the confidence interval for Pedicularis litter) for biomass and N dynamics during decomposition. Significant differences between the two litter types are given (*** $P < 0.001$)

<table>
<thead>
<tr>
<th></th>
<th>Rhinanthus litter</th>
<th>Pedicularis litter</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass</td>
<td>$a$</td>
<td>0.011 ± 0.001</td>
<td>&gt; 0.003 ± 0.001</td>
</tr>
<tr>
<td>(m=0.087)</td>
<td>$b$</td>
<td>0.050 ± 0.004</td>
<td>&gt; 0.024 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>$a+b$</td>
<td>0.061 ± 0.004</td>
<td>&gt; 0.027 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.992</td>
<td>0.953</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$a$</td>
<td>0.009 ± 0.001</td>
<td>&gt; 0.002 ± 0.001</td>
</tr>
<tr>
<td>(m=0.200)</td>
<td>$b$</td>
<td>0.023 ± 0.009</td>
<td>&lt; 0.109 ± 0.010</td>
</tr>
<tr>
<td></td>
<td>$a+b$</td>
<td>0.032 ± 0.009</td>
<td>&lt; 0.111 ± 0.010</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.960</td>
<td>0.889</td>
</tr>
</tbody>
</table>
Figure 2.4 Hemiparasitic litter decomposition dynamics: (a) Percentage litter mass, (b) percentage and (c) absolute N release from decomposing Rhinanthus leaf litter (solid symbols) and Pedicularis plant litter (open symbols) (means ± SE, n = 6). Curves represent modeled data for Rhinanthus leaf litter (solid lines) and Pedicularis plant litter (dashed lines) according to a composite-exponential model (Equation 2.2; see parameters in ). Dotted horizontal lines (c) represent the total of N in the hemiparasite litter.
Chapter 2

2.4 Discussion

2.4.1 Net effect on shoot biomass

Removal of *Rhinanthus* significantly increased total biomass production (including *Rhinanthus*) (+24%), and potential host (total excluding *Rhinanthus*) (+41%), graminoid (+47%) and forb (excluding *Rhinanthus*) (+20%) biomass production in the second summer after the initiation of weeding, while there was no effect during the first summer. A one-year lag in effects on forb and grass biomass has been reported before (Ameloot et al. 2006b); in addition, *Rhinanthus* was initially removed during flowering time and had therefore drained host resources already. The magnitude of the biomass increase is in line with other weeding experiments of *Rhinanthus* spp. (see Ameloot et al. 2005 for a meta-analysis), which reported on average a significant increase in total biomass (+27%) and potential host biomass (total biomass without hemiparasite, +43%).

Effects on non-leguminous forbs were variable in the meta-analysis. In the present study, forb biomass did increase, but less significantly than the other growth forms. When sites were analyzed separately, forb biomass only significantly increased at one *Rhinanthus* site (Rhin-P), where forbs are more abundant than graminoids (Figure 2.5). Graminoids explain most of the aboveground biomass increase, but also forb biomass showed a positive response. In previous weeding studies with *Rhinanthus*, effects on non-leguminous forbs were variable (Ameloot et al. 2005; Mudrak and Leps 2010).
Weeding of *Pedicularis* did not significantly increase total, graminoid or ericaceous aboveground biomass. Little is known about the genus *Pedicularis* and, to our knowledge, no studies exist reporting the effect of *Pedicularis* infection on plant community biomass production. We speculate that litter effects – via an increase in nutrient availability – could have compensated for most of the decrease in community productivity due to parasitism at *Pedicularis* sites, where nutrients limit plant growth more than at *Rhinanthus* sites. Spasojevic and Suding (2011) found the perennial *Castilleja occidentalis* to be associated with an almost twofold increase in productivity, speculating that the positive effect of litter outweighs the effect of parasitism in nutrient-poor systems with long-lived hemiparasites. However, this is not necessarily true at the community level: long-lived hemiparasites may compensate for the negative...
community-level effect of parasitism due to the concentration of nutrients in patches from a relatively large area (Quested et al. 2003b; Spasojevic and Suding 2011). In contrast, it is less likely that the biennial *P. sylvatica* forms long-term nutrient-enriched patches.

The response of aboveground biomass production following weeding of *Rhinanthus* increased in the second summer (2010) compared to the first summer after the start of weeding (2009) (Table 2.3). This was significant for the total and graminoid biomass. At *Pedicularis* sites, weeding had no significant effect on the aboveground biomass. We recall that hemiparasite weeding was initiated in the second half of June, when the hemiparasites were actively growing. It could be that this timing was too late for the weeding treatment to have a (positive) effect on aboveground biomass in 2009. Since there was no negative effect either, we speculate that the biomass loss due to hemiparasite removal was already compensated for by an increased shoot growth of the potential host community.

### 2.4.2 Potential litter effect

Nutrients (NPK) returned to the soil by *Rhinanthus* litter were about 9% of those removed in total vegetation shoot biomass. In contrast, the amounts of N, P and K returned to the soil by *Pedicularis* litter were on average 30, 56 and 72% of those removed by mowing. These results suggest that litter effects on nutrient availability of *Pedicularis* are potentially much more important, at least if nutrient release rates are not much slower compared to *Rhinanthus* litter. Seen from the litterbag experiment, both litter types released nearly half of their initial N content within two months (Figure 2.4b). During an eight-month period, virtually all *Rhinanthus* litter N was released, while
still 41% of *Pedicularis* litter N remained in the litter bags. This indicates that *Pedicularis* litter contains a much more recalcitrant organic fraction compared to *Rhinanthus* litter. This is not surprising, as in our definition of litter (see material and methods) only *Rhinanthus* leaves were considered to form litter, while for *Pedicularis* litter total aboveground plants were considered. Though both litter types have similar C:N ratios, *Pedicularis* litter has a higher lignin concentration. Initial litter C:N ratios (Taylor et al. 1989) and C quality (lignin and carbohydrate concentrations) (Hobbie 1996) are good predictors for litter decomposition rates. Berg et al. (1982) and Berg (2000) found that, while initial litter N content is a good predictor of decomposition in the early stages, the lignin concentration becomes a better predictor at later stages. Indeed, while the initial decay rate \((a+b)\) is already lower for *Pedicularis* litter compared to *Rhinanthus* litter, this difference is more pronounced for the final decay rate \((a)\). Although the percentage of initial litter N released from *Pedicularis* litter drops below that of *Rhinanthus* after two months and does not exceed 60% in the eight-month period (Figure 2.4b), the total N released per m² from the hemiparasitic plant litter remains highest for *Pedicularis* litter over the whole period. This is due to the higher amount of *Pedicularis* litter produced.

Nitrogen released from *Rhinanthus* litter within two and eight months increased from about 4% to 8% of the N removed in total vegetation shoot biomass. In contrast, the amounts of N released from *Pedicularis* litter within two and eight months were higher, but only increased slightly from 14% to 18% of the N removed by mowing. Although the difference between the two hemiparasites is somewhat lowered by the slower N release from *Pedicularis* litter, these results suggest that litter effects on N availability of *Pedicularis* are potentially two to three times more important compared to *Rhinanthus*. 
2.5 Conclusions

Temperate semi-natural grasslands are biodiversity hotspots of global importance (Wilson et al. 2012) and their biodiversity is seriously threatened by increased atmospheric N deposition (Bobbink et al. 2010). Nitrogen addition, and nutrient addition in general, causes strong reductions in species richness due to competitive suppression of forbs and/or ericaceous shrubs by grasses (Silvertown 1980; De Schrijver et al. 2011). The effect of *Rhinanthus* spp. on sward composition is much like the opposite of that observed when soil fertility is increased (Davies et al. 1997); therefore, its deliberate introduction has been suggested as management tool to restore species-rich grassland after the cessation of fertilization (Pywell et al. 2004) as well as a means to reduce the mowing frequency on road verges (Ameloot et al. 2006a). Our results support the idea of using *Rhinanthus* as a management tool, as it had strong negative effects on productivity, mainly of the dominant graminoid component. *Pedicularis*, on the other hand, seems less suited for introduction in the sense that it had no significant effect on vegetation shoot biomass. Moreover, *P. sylvatica* is a species typically lost from oligotrophic meadows when nutrient loading increases or mowing ceases followed by an increase in biomass of stronger competitors (Leps 2005); therefore its introduction in previously fertilized habitats is questionable. Of course, there are other traits that matter in ecosystem restoration such as the attraction of many pollinators in spring by the conspicuous flowers of *Rhinanthus* and *Pedicularis* (personal observation) as well as the temporal variation in species abundances induced by these short-lived hemiparasites in ecosystems dominated by perennial plants. Both hemiparasites have fluctuating cohort abundances that can vary greatly among years (Petru 2005; Ameloot et al. 2006b). This is particularly important in the case of the biennial *Pedicularis* due to
its year-to-year oscillation in seedling recruitment (Petru 2005). In addition, *Pedicularis*
presumably retranslocates nutrients the first year to its tap root, while all nutrients are
released the next year when dying off. Therefore, nutrient recycling events through
*Pedicularis* litter can be subject to large year-to-year variations.

Hemiparasitic plants are known to affect community structure and plant diversity (Press
and Phoenix 2005) and parasitism and litter pathways are held responsible for this (Press
1998). We demonstrated for two short-lived hemiparasites that the relative importance
of litter effects might differ substantially, with higher potential litter effects in the
oligotrophic *Pedicularis* sites compared to the mesotrophic *Rhinanthus* sites. These
findings are in line with the suggestion that the litter pathway becomes more important
when nutrients are more limited (Quested et al. 2003b; Spasojevic and Suding 2011), but
note that differences in site fertility are confounded with the different hemiparasitic and
co-occurring species as well as with the different management regime. Future research
could therefore focus on more hemiparasites along a productivity gradient, preferably at
the same site. *Rhinanthus* had, in contrast to *Pedicularis*, a significant net (negative)
effect on vegetation shoot biomass. We speculate that a strong positive feedback on
nutrient availability and shoot biomass by *Pedicularis* litter is at least partly responsible
for the absence of a net effect on shoot biomass. To separate parasitism effects
unambiguously from litter effects, experiments could add litter to unparasitized plots
and remove litter from parasitized plots.
Effects of *Rhinanthus* and *Pedicularis* on community composition and seedling establishment


**Abstract**

Hemiparasitic plants can profoundly affect the structure of the community in which they occur, mainly due to parasitic suppression of hosts. As a consequence, non-host species have the opportunity to colonize resulting gaps. In contrast to most grassland species, hemiparasites are generally short-lived and can reach high densities; as a consequence, vegetation gaps are left after their death. These gaps form microsites more suitable for seed germination and therefore might increase recruitment of other species. We selected two hemiparasitic plant species from contrasting vegetation types: *Rhinanthus angustifolius* growing in mesotrophic grassland and *Pedicularis sylvatica* growing in oligotrophic heath-grassland. A weeding experiment was set up at six sites in which the hemiparasite was repeatedly removed in half of the plots during three growing seasons. The abundance of individual species was compared between weeded and control plots. After the second growing season, seeds of up to ten species were added. The
number of seedlings in the third year was then compared between weeded and control plots. We found that *Rhinanthus* removal significantly affected the abundance of species relative to control plots, both positively and negatively. *Pedicularis* removal only increased the abundance of some species. Only *Juncaceae* (but no other graminoid families) increased after *Rhinanthus* and *Pedicularis* weeding and there was considerable variation within growth forms. Interestingly, we found indications that species with clonal growth, combining vegetative reproduction with lateral spread, were more often severely parasitized than species without clonal growth. Finally, only half of the sown species successfully established seedlings; hemiparasite removal had a significantly negative effect on seedling number for two of these species. We conclude that effects of hemiparasites on species differ considerably, also within growth forms. Clonal growth emerges as an important plant trait determining vulnerability to hemiparasite attack. Finally, our results suggest that hemiparasitic plants might have a limited positive effect on seedling establishment in these semi-natural grasslands where chances for successful establishment were shown to be low.

### 3.1 Introduction

Hemiparasitic plants, partly carbon autotrophic plants (Těšitel et al. 2010) that depend on host plants for water, mineral and partly carbon provisioning, can profoundly affect plant community structure and diversity (Press and Phoenix 2005). Press (1998) proposed that the net effect of hemiparasites depends on the relative importance of parasitism and litter effects. First, competitive relationships between hosts and non-host species in the plant community may change due to parasitic suppression of the hosts (Gibson and Watkinson 1991; Matthies 1996; Press et al. 1999), if a host preference exists (Gibson and Watkinson 1989; Gibson and Watkinson 1991; Musselman and Press 1995; Cameron 2004). Second, the input of often nutrient-rich and rapidly decomposing hemiparasitic litter (Seel and Press 1993; Pate 1995; Quested et al. 2002) potentially
Species abundance and establishment

increases nutrient cycling and availability (e.g., Bardgett et al. 2006; Ameloot et al. 2008) and thus may stimulate primary production of both host and non-host species.

To study possible effects of hemiparasitic plants on community structure, pot experiments have been conducted to examine parasite-host interactions between hemiparasitic plants and a selection of hosts (e.g., a legume, a grass and a forb). For the well-studied *Rhinanthus minor*, host preference is reported to decrease from legumes over grasses to non-leguminous forbs (e.g., Gibson and Watkinson 1991; Cameron et al. 2006); while for *Pedicularis* spp., host preference appeared to be exactly reciprocal (Hedberg et al. 2005; Ren et al. 2010). Conversely, both observational and experimental field studies using artificial species assemblages (Joshi et al. 2000) or (semi-)natural plant communities (Ameloot et al. 2005; Press and Phoenix 2005) found that *Rhinanthus*, for instance, suppressed total, grass and legume biomass. In contrast, effects on non-leguminous forbs were variable (Davies et al. 1997; Ameloot et al. 2006a; Ameloot et al. 2006b; Mudrak and Leps 2010).

Where pot-experiments allow the inclusion of few species and lack the representativeness to infer *in situ* effects on species composition, field studies generally did not consider the effects of hemiparasitic plants on individual species and focused on growth forms instead (mostly graminoids, legumes and other forbs; but see Mudrak and Leps 2010). Since hemiparasitic plants have been reported to affect members within groups such as grasses and forbs differently (Gibson and Watkinson 1991; Cameron et al. 2006; Mudrak and Leps 2010), more species-level field studies are needed to better understand and predict possible effects of hemiparasitic plants on community composition.
Chapter 3

Hemiparasitic plants can also influence the community composition by increasing establishment opportunities for co-occurring and colonizing species. Gaps appear in the vegetation after the death of the hemiparasite. As a result, suitable microsites (e.g., more light, bare ground) for germination and seedling establishment are created (Joshi et al. 2000; Ameloot et al. 2006b). This expectation can be tested with a seed addition experiment. Reviews on seed addition studies showed that seed limitation – the availability of seeds – is very common in (semi-)natural vegetation (Turnbull et al. 2000). Yet, establishment limitation – the availability of suitable microsites – is the real seed-to-seedling bottleneck for many species and aboveground disturbance (e.g., trampling, mowing) generally has a significantly positive effect on seedling emergence (Clark et al. 2007). Limited knowledge on the effects of the presence of a hemiparasite on seedling establishment of sown species is available. For instance, Pywell at al. 2004) found that the frequency of 6 out of 10 sown forb species was positively correlated with R. minor abundance in the previous year.

Here, we studied how hemiparasites influence other species in the community depending on ‘life stage’. The central question is: how do hemiparasites influence species in the established phase (part I) and species in the establishment phase (part II). We set up a removal experiment in which contrasting hemiparasites, i.e., Rhinanthus angustifolius C.C. Gmel. and Pedicularis sylvatica L. (hereafter referred to as Rhinanthus and Pedicularis), were repeatedly weeded in half of the plots during three years. In part I we aimed to identify species that are suppressed by parasitism and, on the other hand, which non-host species are favored by parasitic suppression of host species. The abundance of individual species was assessed. We hypothesized that (i) graminoids and
Species abundance and establishment

legumes increase most in abundance following weeding of hemiparasitic plants, and (ii) the effects on non-leguminous forbs would be more variable (both positive and negative). In part II we determined the number of established seedlings after seed addition of up to 10 species in both parasitized and weeded plots. We expected to find more seedlings in parasitized plots compared to control plots as a result of decreased establishment limitation.

3.2 Materials and methods

This study was carried out in the same three mesotrophic *Rhinanthus* sites and three oligotrophic *Pedicularis* sites described in chapter 2 (part 2.2.1, Table 2.1).

3.2.1 Hemiparasite removal experiment

Hemiparasite removal in the experiment used in chapter 2 (part 2.2.2) was continued monthly from April to October 2011. In 2009, 2010 and 2011, we estimated species cover in all plots. In 2009 and 2011, directly after species cover estimations, the aboveground biomass of all species was clipped at 2 cm above the soil surface between mid-July and early September, i.e., mimicking the mowing regime of these grasslands. Samples were identified to the species level and sorted (*Poaceae*, *Cyperaceae* and *Juncaceae* were kept as groups) before they were oven-dried (48 h at 70°C) and weighed. *Rhinanthus* sites Rhin-A and Rhin-D were additionally mown in September to correspond with the management, samples were oven-dried and weighed but not sorted. For the population Pedi-H, data on shoot biomass were unavailable for 2009 and were therefore estimated from cover estimates in 2009 based on regressions between cover estimates and shoot biomass at the same site in 2011 ($R^2$ higher than 0.4).
3.2.2 Sowing treatment

In November 2010, i.e., the weeding treatment was already applied for two growing seasons creating variation in the community structure, a sowing treatment was applied to one control and one weeded plot within each block (Figure 3.1). Hence, we created a full factorial design. Sown species were selected from representative species of these communities (Swertz et al. 1996; Zuidhoff et al. 1996) (Table 3.1). For species already present at the sites (underlined in Table 3.1), seeds were collected in the field. Seeds from other species – not present or insufficiently abundant for seed collection – were acquired from Ecoflora, a nursery providing seeds acquired from regional populations (http://www.ecoflora.be). Seedlings of the sown species were counted in all seed addition plots during the summer and autumn of 2011. The maximum number of

![Figure 3.1](image)

**Figure 3.1** The 4 treatments per block of the 3-year weeding experiment: parasitized controls (0), weeding of hemiparasites (-P), seed addition to parasitized plots (0+S) and seed addition to unparasitized plots
seedlings of both surveys was used for further analysis to represent a maximal seedling establishment estimate. *Hieracium* spp. seedlings were identified to the genus level.

**Table 3.1** Number of seeds added per field plot (0.5 x 0.5 m), average germination percentage in open ground determined in a separate trial (n = 3) and the number of viable seeds added per field plot (the product of added seeds and percentage germination in open ground) for seed mixtures used in both vegetation types. Seeds from underlined species were collected in the field, others acquired from Ecoflora (http://www.ecoflora.be). The seed mixtures have two species in common (shaded).

<table>
<thead>
<tr>
<th></th>
<th>Number of seeds added (plot^{-1})</th>
<th>Germination open ground (%)</th>
<th>Viable seeds (plot^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rhinanthus sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lotus uliginosus</em></td>
<td>300</td>
<td>18%</td>
<td>55</td>
</tr>
<tr>
<td><em>Ranunculus acris</em></td>
<td>300</td>
<td>80%</td>
<td>241</td>
</tr>
<tr>
<td><em>Lychnis flos-cuculi</em></td>
<td>2000</td>
<td>57%</td>
<td>1137</td>
</tr>
<tr>
<td><em>Succisa pratensis</em></td>
<td>176</td>
<td>17%</td>
<td>31</td>
</tr>
<tr>
<td><em>Heracleum spondylium</em></td>
<td>71</td>
<td>22%</td>
<td>16</td>
</tr>
<tr>
<td><em>Bellis perennis</em></td>
<td>4369</td>
<td>45%</td>
<td>1981</td>
</tr>
<tr>
<td><em>Lythrum salicaria</em></td>
<td>7627</td>
<td>37%</td>
<td>2797</td>
</tr>
<tr>
<td><em>Lycopus europaeus</em></td>
<td>1931</td>
<td>11%</td>
<td>206</td>
</tr>
<tr>
<td><em>Eupatorium cannabinum</em></td>
<td>2027</td>
<td>39%</td>
<td>784</td>
</tr>
<tr>
<td><em>Valeriana repens</em></td>
<td>898</td>
<td>21%</td>
<td>192</td>
</tr>
<tr>
<td><strong>Pedicularis sites</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lotus uliginosus</em></td>
<td>200</td>
<td>34%</td>
<td>67</td>
</tr>
<tr>
<td><em>Potentilla erecta</em></td>
<td>300</td>
<td>10%</td>
<td>30</td>
</tr>
<tr>
<td><em>Erica tetralix</em></td>
<td>2000</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td><em>Succisa pratensis</em></td>
<td>176</td>
<td>17%</td>
<td>31</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>3010</td>
<td>67%</td>
<td>2027</td>
</tr>
<tr>
<td><em>Hieracium umbellatum</em></td>
<td>1420</td>
<td>53%</td>
<td>748</td>
</tr>
<tr>
<td><em>Hieracium pilosella</em></td>
<td>1974</td>
<td>45%</td>
<td>895</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>218</td>
<td>79%</td>
<td>171</td>
</tr>
<tr>
<td><em>Hypochaeris radicata</em></td>
<td>581</td>
<td>86%</td>
<td>500</td>
</tr>
</tbody>
</table>

At the same time of seed addition in the field, a germination trial in open ground was performed at the lab (outside) to test the viability of the seeds under field-realistic conditions. Seeds were added (50 seeds, n = 3) to 5 cm deep layers of compost in plastic trays that were dug in the ground. Different composts were used for the *Rhinanthus* and *Pedicularis* seed mixtures so that the pH was similar to the soil pH at *Rhinanthus* sites.
(5.2) and Pedicularis sites (4.6) respectively. As none of the collected seeds of Erica tetralix germinated in open ground, seedlings observed in field plots were supposed to result from established individuals and therefore excluded from the analyses.

### 3.2.3 Data analysis

Data for Rhinanthus and Pedicularis sites were analyzed separately. We applied mixed-effects models using the lme4 package in R 2.12.1 (the R Development Core Team 2011). We analyzed the difference in hemiparasite abundance between the years using Tukey’s honestly significant difference post-hoc testing after fitting the model $mass_{HP} \sim year + (1|plot)$, in which $mass_{HP}$ is the shoot biomass of the hemiparasite in unweeded plots, year is a factor with levels ‘2009’, ‘2010’ and ‘2011’, and the random effect $plot$ (18 levels) to account for the repeated estimations within the same plots. We analyzed the effect of hemiparasite weeding on individual species’ biomass response ratio using the model $LN \left( \frac{mass_{2011}}{mass_{2009}} \right) \sim weeding + (1|block)$, in which $mass_x$ is the shoot biomass of an individual species in year $x$, $weeding$ the independent fixed factor with levels ‘control’ and ‘weeded’, and including random intercepts for $block$ (9 levels, 3 at each location). The random effect was added because biomass measurements within the same block are considered non-independent replicates. The mean weeding effect and 95% confidence intervals per species was calculated using Markov Chain Monte Carlo (MCMC) methods (languageR package, Baayen 2011), excluding species with frequencies < 25% (9 out of 36 plots). Because we measured shoot biomass for graminoids only at the family level (Poaceae, Cyperaceae and Juncaceae), cover data were used in a separate analysis to evaluate weeding effects on individual graminoid species. As the hemiparasite abundance in 2011 was too low to detect weeding effects
on cover estimates (Figure 3.2), 2010 cover data were used instead by substituting the dependent variable in the model above with $LN(\text{cover}_{2010}/\text{cover}_{2009})$. Spearman correlations ($r_s$) between weeding effects from cover- and biomass-based models in 2011 ($r_s = 0.45$) and between weeding effects from 2010 and 2011 cover-based models ($r_s = 0.74$) justified this approach.

Similarly, we analyzed the effect of hemiparasite weeding on the number of germinated seeds of individual species using the model $N_{\text{seedlings}} \sim \text{weeding} + (1|\text{location})$, in which $N_{\text{seedlings}}$ is the number of seedlings observed in 2011, $\text{weeding}$ the independent fixed factor with levels ‘control’ and ‘weeded’, and including random intercepts for $\text{location}$. A poisson distribution was used for these count data. The significance level used in hypothesis testing was set to 0.1. Model fits were checked by graphical inspection for normality and homogeneity of residuals.

### 3.3 Results

#### 3.3.1 Hemiparasite abundance and overall effect

Hemiparasite shoot biomass showed considerable interannual variation (Figure 3.2). In control plots at *Rhinanthus* sites, hemiparasite shoot biomass was highest in 2009 (44.7 ± 3.7 g m$^{-2}$), and declined by 26% in 2010 ($P = 0.02$) and 75% in 2011 ($P < 0.001$), relative to 2009. The concurrent effect of *Rhinanthus* removal on the total shoot mass response ratio $LN\left(\frac{\text{mass}_{\text{years}}}{\text{mass}_{2009}}\right)$ was significant in 2010 (+0.24, +27%, $P = 0.001$), but not in 2011 (+0.03, +3%, $P = 0.7$). In control plots at *Pedicularis* sites, hemiparasitic biomass was highest in 2009 (16.5 ± 1.9 g m$^{-2}$), and declined by 34% in 2010 ($P = 0.03$) and 71% in 2011 ($P < 0.001$), relative to 2009. The concurrent effect of *Pedicularis* removal on the
total shoot mass response ratio $LN \left( \frac{mass_{year}}{mass_{2009}} \right)$ was insignificant in both 2010 (+0.13, +14%, $P = 0.4$) and 2011 (+0.15, +16%, $P = 0.3$).

3.3.2 Effects on species abundances

The effects of *Rhinanthus* removal on individual species’ shoot mass response ratios $N \left( \frac{mass_{2011}}{mass_{2009}} \right)$ ranged from -1.08 to +1.36, indicating that the 2011:2009 biomass ratio in weeded plots was between 34% and 390% of that in control plots (Figure 3.3a). Weeding had a significant positive effect on the performance of *Achillea ptarmica* (+1.36, +290%, $P = 0.04$) and *Juncaceae* (+0.31, +36%, $P = 0.06$) relative to unweeded plots, and significantly reduced the performance of *Rumex acetosa* (-0.98, -62%, $P = 0.04$) and *Dactylorhiza maculata* (-0.59, -45%, $P = 0.04$). Unexpectedly, weeding did not significantly increase the performance of *Poaceae* as a group (-0.12, -11%, $P = 0.43$), nor that of the legumes *Lotus uliginosus* (+0.05, +5%, $P = 0.89$) and *Vicia cracca* (+0.61, +84%, $P = 0.33$). When using cover response ratios $LN \left( \frac{cover_{2010}}{cover_{2009}} \right)$ for individual...
graminoid species, weeding had a positive effect on the performance of *Agrostis capillaris* (+0.70, +101%, *P* = 0.005) and *Juncus acutiflorus* (+0.41, +51%, *P* = 0.001) relative to unweeded plots. For *Carex* spp. (-0.34, -29%, *P* = 0.11) and *Scirpus sylvatica* (-0.20, -18%, *P* = 0.46), weeding had a negative yet insignificant effect. Weeding had no effect on *Anthoxanthum odoratum* (*P* = 0.69), *Holcus lanatus* (*P* = 0.60) and *Luzula multiflora* (*P* = 0.99).

**Figure 3.3** The effects of hemiparasite weeding (± 95% confidence bounds) on species’ biomass (ln[biomass$_{2011}$/biomass$_{2009}$]) occurring in *Rhinanthus* sites (a) and *Pedicularis* sites (b). Positive effects indicate that weeding increased the 2011:2009 biomass ratio, and vice versa. Only species that were present in 25% of the plots in both years are shown. Significance levels (*** *P*<0.01, ** *P*<0.05, * *P*<0.1). Species were present at 1, 2 or 3 of 3 locations (dotted, dashed and solid lines, respectively). Types of clonal growth following Hill et al. (2004) are indicated between parentheses: rhizome formation (Rhiz), rooting at nodes (Node), or no clonal growth (0). For graminoid families with a single dominant species the clonal growth of that dominant species is given (†): *Juncus acutiflorus* for *Juncaceae* at *Rhinanthus* sites and *Molinia caerulea* for *Poaceae* at *Pedicularis* sites.
Chapter 3

The effect of *Pedicularis* removal on individual species’ shoot mass response ratios ranged from -0.24 to +1.73, meaning that the shoot mass ratio in weeded plots was between 79% and 564% of that in control plots (Figure 3.3b). Weeding had a positive effect on the performance of *Erica tetralix* (+1.73, 464%, *P* = 0.003) and *Juncaceae* (+1.39, +301%, *P* = 0.005). Again, weeding did not increase the performance of *Poaceae* as a group (-0.24, -21%, *P* = 0.25). When using cover response ratios for individual graminoid species, weeding had a positive effect on the performance of *Juncus acutiflorus* (+0.54, +72%, *P* = 0.01) and – though not significant – on *Agrostis canina* (+0.44, +55%, *P* = 0.13). Weeding had a negative but insignificant effect on *Carex* spp. (-0.18, -0.16%, *P* = 0.42) and no effect on *Molinia caerulea* (*P* = 0.66) and *Luzula multiflora* (*P* = 0.65).

Interestingly, species that increased their abundance after hemiparasite removal all showed clonal growth combined with lateral spread (Hill et al. 2004) such as rhizome formation (*Achillea ptarmica, Agrostis capillaris, Juncus acutiflorus*), stolon formation (*Agrostis canina*) or creeping and rooting at nodes (*Erica tetralix*). In contrast, species that decreased after weeding did not possess any type of clonal growth (*Dactylorhiza maculata, Rumex acetosa*).

### 3.3.3 Effects on seed germination

Four out of ten species (*Valeriana repens, Eupatorium cannabinum, Lycopus europaeus* and *Lythrum salicaria*) did not germinate at all at *Rhinanthus* sites and another one (*Bellis perennis*) germinated only in 1 of 18 plots. The remaining five species germinated in at least 10 of 18 plots. The effect of *Rhinanthus* removal on the number of seedlings the year after seed addition was negative for *Lychnis flos-cuculi* (-0.4, *P* = 0.01) and
Species abundance and establishment

*Succisa pratensis* (-0.52, *P* = 0.09), while weeding had no significant effect on *Lotus uliginosus* (*P* = 0.21), *Ranunculus acris* (*P* = 0.60) and *Heracleum sphondyllum* (*P* = 0.83) (Figure 3.4a).

At *Pedicularis* sites, two out of the seven considered species (*Achillea millefolium* and *Plantago lanceolata*) did not germinate in the field while *Lotus uliginosus* germinated only in 3 out of 18 plots. The remaining species germinated in at least 7 of 18 plots. The effect of *Pedicularis* removal on the number of seedlings the year after seed addition was negative for *Hieracium* spp. (-1.15, *P* = 0.01) and *Succisa pratensis* (-0.44, *P* = 0.01), while weeding had no significant effect on *Hypochaeris radicata* (0.55, *P* = 0.18) and *Potentilla erecta* (0.38, *P* = 0.13) (Figure 3.4b).

### 3.4 Discussion

#### 3.4.1 Temporal variation of hemiparasite abundance

There was considerable interannual variation in the abundance of *Rhinanthus* and *Pedicularis*. Both hemiparasites performed best in 2009, declined by ca. 30% in 2010 and ca. 73% in 2011 relative to 2009. The considerable temporal and spatial variation of *Rhinanthus* and *Pedicularis* abundances is probably related to their short-lived life strategy (Petru 2005; Ameloot et al. 2006b). Ameloot et al. (2006b) concluded that early spring drought was the main reason for population collapse of the annual *R. angustifolius*. This could explain the collapse of *Rhinanthus* in 2011 relative to previous years: the spring of 2011 was the third driest spring on record (since 1833) in central Belgium (71 mm rainfall compared to 188 mm normal) (Royal Meteorological Institute of Belgium). The spring of 2010 was also remarkably dry with no precipitation events between Apr-8 and Apr-29, when *Rhinanthus* seedlings and *Pedicularis* plants emerged.
We conclude that spring drought is likely the main reason for the year-to-year difference in the hemiparasite abundances we observed.

The low abundance of hemiparasites in 2011 raises the question whether we can use biomass data of this year to evaluate the effect of hemiparasites on species abundances and germination success. While it is likely that effects are less pronounced due to the low hemiparasite abundance in unweeded plots, we still expected to see cumulative effects of the two years of continued hemiparasite removal. For instance, Ameloot et al. (2006b) found that grass and forb cover were related to *Rhinanthus* cover in the previous year (rather than in the same year) in similar grassland types as in the present study, indicating that vegetation responses lag behind hemiparasite abundance changes.

### 3.4.2 Effects on individual species

**Winners and losers**

At *Rhinanthus* sites, we identified both winner and loser species as a result of hemiparasite weeding. At *Pedicularis* sites we also identified winner species, but none of...
the other species declined in biomass following hemiparasite removal. Species that increase their shoot biomass after hemiparasite removal were most likely severely parasitized before: resource allocation to the shoot is decreased by hemiparasitic infection (Graves 1995), and is expected to recover after removal of the hemiparasite. Species that show no or negative effects are most likely unfavourable hosts. At *Rhinanthus* sites, where the total shoot biomass is relatively high (362 ± 72 g m\(^{-2}\) in control plots in 2011), loser species might be outcompeted by one or more winner species due to competition for light. Released from parasitic infection, the winner species may allocate relatively more resources to their shoots, which is a key factor determining light competition (Pan et al. 2011). The more open vegetation structure and low total shoot biomass at *Pedicularis* sites (176 ± 22 g m\(^{-2}\) in control plots in 2011), in contrast, suggests that competition for light is probably less important there and could explain why none of the species was negatively affected by the weeding treatment. Hautier et al. (2009) support this idea, by showing that competition for light causes biodiversity loss after a fertilizer-induced biomass increase, whereas there was no significant light limitation in unfertilized plots. In a weeding experiment with *Rhinanthus minor*, Mudrak and Leps (2010) found *Ranunculus repens* to be associated with plots without *Rhinanthus*, while *R. acris* was intermediate between weeded and control plots. The ranking of the effects of *Rhinanthus* weeding on both *Ranunculus* species (Figure 3.3a) in the present study, showed the same trend, though weeding effects were not significant. *Lychnis flos-cuculi* (de Hullu 1984 in Ameloot 2007, but see ter Borg 1972) and *Rumex acetosa* (Cameron et al. 2006) were reported as poor host species for *Rhinanthus* spp. and profited most from hemiparasite weeding in our study. The family of *Orchidaceae* was reported to be strictly avoided by *Rhinanthoidea* (Weber 1976). Our
results are in agreement with this hypothesis: the only orchid, *Dactylorhiza maculata*, decreased significantly in abundance after weeding of *Rhinanthus*.

**Variable effects within graminoids**

*Juncaceae* clearly won after hemiparasite removal in both studied vegetation types. *Poaceae* and *Cyperaceae*, on the other hand, showed no significant effects as a group. However, at the species level, there was considerable variation in the cover of these taxonomic groups. Within *Juncaceae*, *Juncus acutiflorus* – but not *Luzula multiflora* – showed a significant response to hemiparasite removal in both vegetation types. Within *Poaceae* at *Pedicularis* sites, *Agrostis canina* – though only marginally significant – responded positively to weeding, while *Molinia caerulea* did not. Within *Poaceae* at *Rhinanthus* sites, *Agrostis capillaris* is positively affected by weeding, while *Anthoxanthum odoratum* and *Holcus lanatus* are not. In another removal experiment, with *Rhinanthus minor* (Mudrak and Leps 2010), *Agrostis capillaris* was associated with *Rhinanthus* removal, while *Holcus mollis* was associated with parasitized plots. Similarly, Hautier et al. (2010) showed that *Rhinanthus alectorolophus* performed less when grown with *A. odoratum* and *H. lanatus* than expected based on the absolute growth rates of these grasses. In the same study, *R. alectorolophus* growing with *A. capillaris* performed – as with most other studied grasses – as expected from its absolute growth rate. Hautier et al. (2010) suggested that grasses may differ in their resistance to parasitism. Our results corroborate these findings. Moreover, we agree with the suggestion postulated by Mudrak and Leps (2010) that the net effect on diversity depends on the relative sensitivity of individual dominants, co-dominants and subordinate species in the community and on the ability of resistant or tolerant species to take advantage, when the sensitive dominants are suppressed.
**Defence mechanisms**

Species differ in their ability and strategies to prevent the formation of successful parasite-host connections (*haustoria*). Species showing a strong defensive response such as *Plantago lanceolata* – host cells surrounding the invading *R. minor* tissue were fragmented or disintegrated preventing hemiparasite access to the stele (Cameron et al. 2006; Rümer et al. 2007) – are considered poor hosts and thus are not expected to increase hemiparasite removal. In contrast, species showing no or little defensive response such the preferred host *Vicia cracca* (Rümer et al. 2007), are expected to show the highest increase after hemiparasite removal. In the present study, both species showed no significant response to hemiparasite removal, but the mean effect of weeding on *P. lanceolata* was intermediate (-0.30, -26%, *P* = 0.54), suggesting its non-host status, while the mean effect on *V. cracca* was the second highest (+0.61, +84%, *P* = 0.33), suggesting its status of preferred host. At least our results are not in disagreement with the reported defence strategies. Both *Poaceae* species studied by Rümer et al. (2007) reacted to *R. minor* attacks with strong cell lignifications, but differed in extent of lignification. This might explain the contrasting results we found within this family.

**Clonality hypothesis**

We define clonal growth as vegetative reproduction combined with lateral spread (*sensu* Hill et al. 2004), e.g., through the presence of rhizomes or stolons, and creeping species rooting at nodes. Ramets can belong to an extensive interconnected network of seemingly separate individuals. Once a parasite-host connection is established, the hemiparasite can drain resources from the network to which the clonal plant belongs. Species that significantly increased their biomass following hemiparasite removal generally possessed clonal growth (following Hill et al. 2004), while those that decreased
did not (Figure 3.3). The graminoids that increased in abundance by hemiparasite removal have rhizomes (*Agrostis capillaris, Juncus acutiflorus*) or stolons (*Agrostis canina*), while those that were unaffected by weeding (*Holcus lanatus, Anthoxanthum odoratum, Molinia caerulea* and *Luzula multiflora*) showed no clonal growth. Though this ‘clonality hypothesis’ needs broader testing, our results suggest – for the first time – that clonality might indeed be a key trait determining species’ susceptibility to hemiparasitic infection.

### 3.4.3 Germination success

Not all sown species were able to germinate in the field. In the more productive *Rhinanthus* sites, a smaller proportion of species germinated compared to the more open vegetation at *Pedicularis* sites. It should be noted, however, that the composition of the sown seed mixture was different in both vegetation types (only two species were common to both applied seed mixtures). *Rhinanthus* and *Pedicularis* removal had significantly negative effects on the germination success of two out of five and two out of four species, respectively (Figure 3.4). For the species that germinated successfully in the field, our results corroborated the hypothesis that hemiparasitic plants can enhance the germination success of other plant species. Hautier et al. (2009) found that seed mortality increased with light limitation. However, our hemiparasite removal experiment does not allow to distinguish between effect on light availability (‘gap’ formation after the death of the hemiparasite and decreased total aboveground biomass) and effects on the availability of bare ground patches (‘gap’ formation). Interestingly, Pywell et al. (2004) found that the frequency of *Rhinanthus minor* in the previous year explained variation in richness and frequency of sown species, whereas
Species abundance and establishment

*R. minor* frequency in the current year did not. This can explain why we see a negative effect of weeding on the number of seedlings of sown species despite of the population collapse of both hemiparasite species in the year of germination.

### 3.4.4 Where to go from here

In accordance with previous studies, our results suggest that hemiparasites are important mediators of vegetation structure by changing the relative abundances of host and none host species as well as creating opportunities for seedling establishment. In line with the results of Mudrak and Leps (2010), we showed that more species-level field studies are required because we found several contrasting effects of hemiparasites on members of the same growth form or family. This implies that, depending on species composition, effects on community structure and species diversity can be adverse. For the first time we hypothesize that, in addition to the lack of a sound defensive response, also clonal growth might be an important plant trait determining vulnerability to hemiparasite attack. To find evidence for this hypothesis, future research could set up an experiment in which the impact of hemiparasites is compared between an intact clonal structure and clonal structures of the same species in which the conducts between individual ramets are interrupted.
Effects of *Rhinanthus* and *Pedicularis* litter on gross N transformation rates in the soil


**Abstract**

Hemiparasitic plants accumulate nutrients in their leaves and therefore produce high-quality litter with faster decomposition and nutrient release rates compared to litter of co-occurring species. Higher levels of plant-available nitrogen (N) in the presence of hemiparasitic plants have been attributed to this ‘litter effect’, but effects on N dynamics in the soil remain unstudied. We tested the hypothesis that litter of *Rhinanthus angustifolius* and *Pedicularis sylvatica* increase N transformations in the soil more than a litter mix of co-occurring species. We expected the litter effect to be higher in the mesotrophic *Rhinanthus* soil compared to the oligotrophic *Pedicularis* soil. Gross N transformation rates were quantified using a $^{15}$N tracing modeling approach. Differentially $^{15}$N labeled NH$_4$Cl + KNO$_3$ was added to two soils with three treatments (control, soil amended with *Rhinanthus* or *Pedicularis* litter, soil amended with a litter mix of co-occurring non-parasitic
species in a laboratory incubation experiment. The concentration and $^{15}$N enrichment of NH$_4^+$ and NO$_3$ were measured at six time steps within one or two weeks (depending on the soil) after label addition. In general, *Rhinanthus* and *Pedicularis* litter both increased the turnover of inorganic N in the soil more than litter of co-occurring species. Relative to the litter mix, addition of *Rhinanthus* litter increased the net flux from organic N to NH$_4^+$ by 61% and net (autotrophic) nitrification by 80%. Addition of *Pedicularis* litter increased the net flux from organic N to NH$_4^+$ by 28% relative to addition of litter from co-occurring species. Our results support the hypothesis that litter from hemiparasitic plants increases soil N availability more than litter from co-occurring species. This litter-induced augmentation in soil fertility provides – in addition to the parasitic suppression of hosts – a second potentially important pathway by which hemiparasitic plants impact on community composition.

### 4.1 Introduction

The net effect of hemiparasitic plants on plant community structure and diversity results from both parasitism and litter pathways (Press 1998; Spasojevic and Suding 2011). The parasitism pathway refers to direct negative effects of hemiparasitic plants on host species and indirect positive effects on non-host species. Most parasitic plants are generalists, but show high levels of host preference (Press and Phoenix 2005). Therefore, parasitism changes the competitive relations between preferred and non-preferred hosts in the vegetation with possible effects on diversity (Gibson and Watkinson 1991; Matthies 1996; Press et al. 1999). For example, the decrease of total, graminoid and legume biomass in grasslands infected with *Rhinanthus* spp. is thought to alter the species composition in favor of non-leguminous forbs and to increase the local diversity (Gibson and Watkinson 1991; Davies et al. 1997; Ameloot et al. 2005). The litter pathway operates via effects on nutrient cycling. Hemiparasitic plants accumulate
Gross N transformation rates

nutrients in their tissues, which is thought, in part, to be a result of their high transpiration rates (Gauslaa 1990; Gauslaa and Odasz 1990; Pate 1995; Phoenix and Press 2005) and therefore produce litter with high decomposability (Seel and Press 1993; Press 1998; Press et al. 1999; Quested et al. 2002; Quested et al. 2003a). There is limited evidence that hemiparasites can increase the amount of nitrogen (N) inputs to the soil (Quested et al. 2003a; March and Watson 2010), increase net N mineralization (Bardgett et al. 2006), increase the amount of plant-available N (Ameloot et al. 2008), and enhance plant growth (Quested et al. 2003b). In grassland ecosystems, this increase in plant-available N (and likely other nutrients) potentially increases graminoid biomass and therefore might decrease diversity and change community composition as generally observed in nutrient addition experiments (Silvertown et al. 2006; Hejcman et al. 2007; De Schrijver et al. 2011). All together, parasitism is expected to decrease productivity and to impact on diversity, either positively or negatively, depending on host preference, while litter effects may increase productivity and have weaker impacts on diversity (Spasojevic and Suding 2011).

While the impact of hemiparasites on community structure is well-studied (see the reviews of Ameloot et al. 2005; Press and Phoenix 2005), studies looking at the litter pathway are more scarce. Press (1998) suggested that litter effects of hemiparasites should be investigated both in field and microcosm, through simple species manipulation experiments, coupled to litter and tracer studies. The Quested et al. studies (2002; 2003a; 2003b; 2005) used litter in field and mesocosm experiments and found that litter of the subarctic Bartsia alpina increased N inputs to the soil, released N faster than co-occurring species and enhanced plant growth more than litter of non-
parasitic species. March and Watson (2007; 2010) found that the mistletoe *Amyema miquelii* increased both litterfall and annual litter N (x1.65), phosphorus (P, x3) and potassium (K, x8.5) returns in temperate eucalypt forest. Ameloot et al. (2008) introduced *Rhinanthus minor* in temperate grasslands and compared the dilution of added $^{15}$N tracer in the soil between plots with and without *R. minor*. They found added $^{15}$N in the mineral soil N pool to be more diluted in parasitized plots, indicating increased N availability by *R. minor*. The authors discussed several possible reasons for this increase in N availability, of which one is hemiparasitic litter inputs. In an observational study, Spasojevic and Suding (2011) associated *Castilleja occidentalis* presence with higher productivity and foliar N concentrations in co-occurring species in alpine tundra. Based on decomposition trials, in which *C. occidentalis* (alone and in mixtures) lost N much faster compared to the tested co-occurring species, Spasojevic and Suding (2011) speculated that litter effects outweigh parasitism effects. However, the authors found no higher soil inorganic N pool associated with *C. occidentalis*. They concluded that future research should examine N cycling in more detail.

Net N mineralization (gross N mineralization minus gross N immobilization) measurements have long been the fundamental tool in N-cycling research. Plants are considered to lose the competition for NH$_4^+$ from microbes; therefore, gross mineralization was believed to fulfill microbial demands in the first place, before being available for plant uptake. Research findings of the last decades pointed towards a new paradigm of the N cycle in which plant uptake competes with microbial immobilization (Schimel and Bennett 2004), especially in low-N environments. Therefore, beside net mineralization, also gross mineralization should be assessed. This is commonly done by
Gross N transformation rates using analytical equations with data from $^{15}$N dilution experiments (e.g., Kirkham and Bartholomew 1954), allowing determination of the total gross production and consumption of the labeled pool (either $\text{NH}_4^+$ or $\text{NO}_3^-$); gross mineralization is assumed to be equal to gross total $\text{NH}_4^+$ production. Major drawbacks of this method are that process-specific transformation rates cannot be determined (Schimel 1996), and that remineralization of added $^{15}$N is assumed to be negligible (Hart et al. 1994). Numerical methods, in contrast, allow estimation of several simultaneously occurring gross N transformations (Myrold and Tiedje 1986; Mary et al. 1998), at least when model parameters are optimized using a robust technique (Müller et al. 2007). Therefore, they are the best available tool to study the N cycle processes in detail.

In this chapter, we aimed at better understanding the litter effect of hemiparasitic plants on soil nitrogen availability. Therefore, we estimated gross N transformation rates in the soil using a numerical data analysis based on a $^{15}$N tracing model (Müller et al. 2007). For two contrasting hemiparasites, we compared N transformations in (i) control soil, (ii) soil amended with a litter mix of co-occurring non-parasitic species and (iii) soil amended with hemiparasitic litter. We selected two hemiparasitic species growing in vegetation types with contrasting nutrient dynamics: *Rhinanthus angustifolius* C.C. Gmel. growing in mesotrophic grasslands and *Pedicularis sylvatica* L. growing in oligotrophic heath-grassland (hereafter *Rhinanthus* and *Pedicularis*). The first hypothesis we tested is that hemiparasitic litter addition increases gross soil N transformation rates more than addition of a litter mix of co-occurring species. The second hypothesis we tested is that the relative effect of hemiparasitic litter on gross soil N transformation rates is higher in the oligotrophic *Pedicularis* soil compared to the mesotrophic *Rhinanthus* soil.
4.2 Materials and methods

This study we selected one mesotrophic *Rhinanthus* site (Rhin-D) and one oligotrophic *Pedicularis* site (Pedi-H) described in chapter 2 (part 2.2.1, Table 2.1).

4.2.1 Litter collection

Withering *Rhinanthus* and *Pedicularis* plants were collected from both sites at the end of June 2010. Since the management is inherently connected to the presence of the hemiparasites, only the part of the plant that returns to the soil as litter under the management regime was used in our experiment: *Rhinanthus* leaves as they fall of before mowing removes the withered stems, and total *Pedicularis* shoots as only the tips are removed by mowing. Mixtures of other species were gathered by clipping total shoot biomass within a randomly selected plot (1 m²) from which the hemiparasite was removed. This was done mid July (Rhin-D) or mid-august (Pedi-H) prior to the regular mowing management took place. The four litter types were oven-dried in the laboratory (25°C) and ground with an ultra centrifugal mill (mesh size 2 mm) (ZM200, Retsch, Germany).

4.2.2 Soil collection and preparations

One week prior to $^{15}$N label additions, fresh mineral soil (5-15 cm) was collected directly under the sod at both sites from three randomly selected 20x20 cm plots. Additionally, three soil cores were taken for determination of bulk density. Roots were sorted out by hand in the laboratory. For each soil type 99 polypropylene containers (180 ml, r = 2.5 cm, h = 10 cm) were weighed, filled with 60 g fresh soil, and weighed again. A third of the containers were amended with 100 mg hemiparasitic litter, another third were amended with 100 mg non-parasitic litter mix and the remaining third served as
Gross N transformation rates

unamended controls (Figure 4.1). All containers were mixed with a spoon after which soil was pressed to a height of 1.9 cm (Pedi-H) or 3.2 cm (Rhin-D) to achieve field bulk density. Containers were weighed and covered with pierced parafilm (7 pinholes) to allow gas exchange but limit evaporation losses. Containers were pre-incubated for one week in a dark room (20°C) to recover after disruption. Every two days parafilm was removed, containers were weighed and distilled water was dripped onto the soil to compensate evaporation losses; that way, field gravimetric moisture content at the time of soil collection was maintained for Rhin-D (51%) and Pedi-H (38%). Meanwhile, we removed seedlings that germinated from the seed bank. One container of each treatment was extracted with 120 mL 1 M KCl at six time steps ($t_1$, $t_2$, ...) ($n = 3$ for $t_{1-4}$; $n = 2$ for $t_5-6$).

Figure 4.1 Experimental setup (a) and picture (b) of the incubation containers. To half of the containers a 1.4 mL solution of $^{15}$NH$_4$Cl + KNO$_3$ was added, to the other half NH$_4$Cl + K$^{15}$NO$_3$. Treatments are 60 g fresh soil (c), 60 g fresh soil + 100 mg non-parasitic litter mix (+m) and 60 g fresh soil + 100 mg hemiparasitic litter (+p). Containers were extracted with 120 mL 1 M KCl at six time steps ($t_1$, $t_2$, ...) ($n = 3$ for $t_{1-4}$; $n = 2$ for $t_5-6$)
in the form of NO$_2^-$ after reduction in a Cd-Cu column followed by the reaction of the NO$_2$ with N-1-naphthylethlenediamine to produce a chromophore. The NO$_3^-$ results were corrected for NO$_2^-$ present in the soil samples. To detect significant trends in NH$_4^+$ and NO$_3^-$, linear regression was performed in SPSS 21.

**4.2.3 $^{15}$N tracing experiment**

The $^{15}$N label was introduced by adding a NH$_4$Cl-KNO$_3$ solution, in which one of the two N moieties was $^{15}$N-labelled (99 atom% excess), with the ‘mirrored’ moiety at natural abundance. Application rates of NH$_4^+$ and NO$_3^-$ (Table 4.1) were about 20% of the standing pool measured in the test samples, so that theoretical $^{15}$N enrichments for NH$_4^+$ and NO$_3^-$ were between 15% and 33% after label application. The added N species were dissolved in a 1.4 mL solution to each container by inserting a spinal needle (BD, 18G x 90 mm) in the soil through a template with 7 evenly spaced holes, releasing 0.2 mL of the solution while withdrawing the needle through the soil. This is essential for a

### Table 4.1

Added amounts of $^{15}$N label applied as a 1.4 mL solution of either $^{15}$NH$_4$Cl + KNO$_3$ or NH$_4$Cl + $^{15}$KNO$_3$ and C:N as well as N:P ratios (mean ± SE) of the two soils and four litter types. Rhin-D: Rhinanthus site ‘Doode Beremde’; Pedi-H: Pedicularis site ‘Hooiput’

<table>
<thead>
<tr>
<th>Label addition NH$_4^+$-N/NO$_3^-$-N (µg container$^{-1}$)</th>
<th>Mesotrophic Rhinanthus soil (Rhin-D)</th>
<th>Oligotrophic Pedicularis soil (Pedi-H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control treatment</td>
<td>10.0/36.6</td>
<td>10.0/1.0</td>
</tr>
<tr>
<td>Litter mix treatment</td>
<td>10.0/18.1</td>
<td>7.2/1.0</td>
</tr>
<tr>
<td>Hemiparasitic litter treatment</td>
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<td>3.7/1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C:N</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
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<td>24 ± 1</td>
</tr>
<tr>
<td>Litter mix</td>
<td>36 ± 2</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>Hemiparasitic litter</td>
<td>25 ± 1</td>
<td>26 ± 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N:P</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>8 ± 1</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Litter mix</td>
<td>7 ± 1</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Hemiparasitic litter</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>
uniform distribution of the added 15N label (Hart et al. 1994). Soil temperature (20°C) and moisture content were kept constant during the entire experiment. Three containers per treatment (two for the last two time steps) were extracted 0.25, 1, 4, 24, 72 and 168 h (Rhin-D soil) or 0.25, 4, 24, 72, 168 and 312 h (Pedi-H soil) after label addition with 120 mL 1 M KCl and shaken for 1 h. The NH$_4^+$ and NO$_3^-$ concentrations in the extract were determined as above. The $^{15}$N contents of NH$_4^+$ and NO$_3^-$ were analyzed after conversion to N$_2$O using a trace gas preparation unit (ANCA-TGII, PDZ Europa, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, SerCon, UK). NH$_4^+$ was converted by adding MgO to soil extracts and absorbing NH$_3$ into H$_2$SO$_4$, after which N$_2$O was produced by reaction with NaOBr (Hauck 1982; Saghir et al. 1993). NO$_3^-$ was reduced by Cd-Cu at pH 4.7 to produce NO$_2^-$ and NH$_2$OH as intermediates of N$_2$O (Stevens and Laughlin 1994). Whenever NH$_4^+$ or NO$_3^-$ concentrations in the KCl extract were too low, they were spiked with an NH$_4$Cl or KNO$_3$ solution at natural abundance.

4.2.4 $^{15}$N tracing model

A numerical $^{15}$N tracing model was used to quantify multiple gross N transformation rates for each treatment. The $^{15}$N tracing model was originally described by Müller et al. (2004). Here, we applied a modified version that relies on a Markov chain Monte Carlo algorithm for parameter optimization (Müller et al. 2007; Rütting and Müller 2007). While analytical equations only quantify the total gross production and consumption of the labeled pool (Schimel 1996; Rütting et al. 2011), this model enables to simultaneously quantify gross rates for a variety of N transformations described either as zero or first order kinetics. This is done by minimizing the misfit function (model optimization) in the form of a quadratic weighted error between the observed data and
the model output. Therefore, the average and standard error of the measured soil \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) concentrations and their respective \( ^{15}\text{N} \) enrichments are used. The model optimization results in a probability density function for each model parameter, from which average parameter values and standard errors (SE) are calculated (Rütting and Müller 2007; Staelens et al. 2011). The optimization algorithm was programmed in MatLab (Version 7.11, The MathWorks Inc.). This algorithm called the \( ^{15}\text{N} \) tracing model, which was separately setup in Simulink (Version 7.6, The MathWorks Inc.).

The three main N pools considered in the tracing model (Figure 4.2) were organic N (\( N_{\text{org}} \)) in soil organic matter (SOM) and litter, as well as extractable ammonium (\( \text{NH}_4^+ \)) and nitrate (\( \text{NO}_3^- \)). For the Pedi-H soil, we included an additional pool of fixed ammonium (\( \text{NH}_4^+_{\text{fix}} \)), accounting for non-extractable \( \text{NH}_4^+ \)-N which is assumed to be fixed quickly to clay-humus complexes (Müller et al. 2004; Röing et al. 2006; Huygens et al. 2007; Russow et al. 2008; Rütting et al. 2010). The rapid fixation of \( \text{NH}_4^+ \) was indicated by the rather low recovery (70 ± 8 %) of \( ^{15}\text{NH}_4^+ \) 15 minutes after label addition. In contrast to the Pedi-H soil, 94 ± 14 % of the added \( ^{15}\text{NH}_4^+ \) was recovered after 15 minutes in the Rhin-D soil.

Several modifications in kinetic settings, considered N pools and included N transformations were tested to identify the model that best described the measured soil mineral N concentrations and \( ^{15}\text{N} \) contents, governed by the Akaike Information Criterion (AIC). A model with a smaller AIC is more likely to be correct and, hence, only modifications decreasing the AIC value were considered for the final data analysis (Burnham and Anderson 2002). Starting with a basic setup – including only \( M_{\text{Org}}, I_{\text{NH}_4}, \)
Gross N transformation rates

Figure 4.2 Nitrogen (N) pools and transformations considered in the $^{15}$N tracing model that was used for data analysis. Included pools are organic N ($N_{org}$) in soil and litter, extractable ammonium ($NH_4^+$) and nitrate ($NO_3^-$) and fixed ammonium ($NH_4^{+fix}$, *Pedicularis* soil only). Fluxes are mineralization of organic N to ammonium ($M_{Norg}$), immobilization of ammonium to organic N ($I_{NH4}$), oxidation of ammonium to nitrate ($O_{NH4}$), oxidation of organic N to nitrate ($O_{Norg}$), immobilization of nitrate to organic N ($I_{NO3}$), fixation of ammonium ($F_{NH4}$) and release of fixed ammonium ($R_{NH4f}$).

$O_{NH4}$ and $I_{NO3}$ – model parameters were added or omitted one by one to examine whether simpler models could describe the measured N dynamics and to assess the robustness of the obtained gross N fluxes (Staelens et al. 2011; Nelissen et al. 2012). To allow easy comparison between treatments, the same set of pools and transformations was used in all three treatments within both soils. In the final model for the Rhin-D soil, three N pools and five transformations were retained (Figure 4.3); for the Pedi-H soil we selected four N pools and seven transformations. The transformations that were not considered in the final model, based on the AIC, were likely not occurring in the soils and hence the gross rates can be assumed to be zero. The initial (i.e., at $t = 0$ h) size and $^{15}$N
content of the NH$_4^+$ and NO$_3^-$ pools were obtained by backwards extrapolation of the first two time steps (0.25 and 1 or 4 h data) (Müller et al. 2004). The initial value of the NH$_4^+$ pool was calculated according to Münchmeyer (2001). Based on the final kinetic settings and model parameters, mean gross N fluxes were calculated by integrating the rates over the total period and subsequent division by the total time (Rütting and Müller 2007; Staelens et al. 2011; Nelissen et al. 2012). Because of the high number of iterations of the $^{15}$N-tracing model, statistical tests are inappropriate for the comparison of results. However, an alternative to a test for significant differences at $\alpha = 0.05$ is to test whether the 85% confidence intervals (85% CI) overlap (Payton et al. 2000; Rütting et al. 2010).

4.3 Results

4.3.1 N pool sizes and $^{15}$N enrichment

In the Rhin-D control soil, NO$_3^-$ was the dominant mineral N form in the soil solution, with an average concentration of 5.2 ± 0.2 μg NO$_3^-$-N g$^{-1}$ soil compared to 0.91 ± 0.05 μg NH$_4^+$-N g$^{-1}$ soil over the entire incubation (Figure 4.3a). Ammonium concentrations were similar in both litter-amended soils, whereas NO$_3^-$ concentrations were ten and seven times smaller compared to the control soil in the litter mix (Figure 4.3b) and hemiparasitic litter treatments (Figure 4.3c), respectively. In the Pedi-H control soil, NH$_4^+$ was the dominant mineral N form in the soil solution: on average 4.68 ± 0.08 μg NH$_4^+$-N g$^{-1}$ soil compared to only 0.05 ± 0.01 μg NO$_3^-$-N g$^{-1}$ soil (Figure 4.3d). Nitrate concentrations were similar in both litter-amended soils, whereas NH$_4^+$ concentrations were two and twelve times smaller compared to the control soil in the litter mix (Figure 4.3e) and hemiparasitic litter treatments (Figure 4.3f), respectively. The NH$_4^+$ and NO$_3^-$
Figure 4.3 Net (black) and gross (grayed out) N transformation rates (μg N g$^{-1}$ day$^{-1}$) between the different N pools (boxes) in Rhin-D soil (a) and Pedi-H soil (b) without litter addition (left), amended with a non-parasitic litter mix (middle), and amended with hemiparasitic litter (right). The arrow widths of the net transformation rates are drawn to scale within both soil types for ease of comparison between the three treatments. Skew lines between white and black parts of a pool’s box indicate that this pool increased during the incubation period.
pools were not static throughout the experiment (data not shown). In the different Rhin-D treatments, \(\text{NH}_4^+\) was stable, while \(\text{NO}_3^-\) increased by 0.42 \(\mu\text{g N g}^{-1} \text{ soil d}^{-1}\) in the control soil \((t = 2.72, P = 0.05)\). NO\(_3^-\) concentrations did not change significantly in the litter mix treatment, and increased by 0.20 \(\mu\text{g N g}^{-1} \text{ soil d}^{-1}\) in the hemiparasitic litter treatment \((t = 3.98, P = 0.02)\). On the contrary, in all the Pedi-H soil treatments, the NO\(_3^-\) concentration was stable and negligible, while \(\text{NH}_4^+\) increased by 0.43 \(\mu\text{g N g}^{-1} \text{ soil d}^{-1}\) in the control soil \((t = 9.67, P < 0.001)\), by 0.21 \(\mu\text{g N g}^{-1} \text{ soil d}^{-1}\) in the litter mix treatment \((t = 4.71, P = 0.003)\) and by 0.05 \(\mu\text{g N g}^{-1} \text{ soil d}^{-1}\) in the hemiparasitic litter treatment \((t = 4.71, P = 0.005)\). Overall, the modeled \(\text{NH}_4^+\) and \(\text{NO}_3^-\) concentrations fitted the measured concentrations well (see appendix B); in the hemiparasitic litter treatments, the modeled \(\text{NO}_3^-\) concentration in the Rhin-D soil and the modeled \(\text{NH}_4^+\) concentration in the Pedi-H soil had not increased sufficiently at the last time step.

In all the Rhin-D soil treatments, the observed enrichment of \(^{15}\text{N}\) in the \(\text{NH}_4^+\) pool after label addition showed a similar fast asymptotic decline (Figure 4.4a-c). In contrast, the enrichment of \(^{15}\text{N}\) in the \(\text{NO}_3^-\) pool showed a slower asymptotic decline and the pace of this decline increased from the control treatment over the litter mix treatment to the hemiparasitic litter treatment (Figure 4.4d-f). In the Pedi-H soil treatments, the observed enrichment of \(^{15}\text{N}\) in the \(\text{NH}_4^+\) pool after label addition declined with increasing rate from the control treatment over the litter mix treatment to the hemiparasitic litter treatment (Figure 4.5a-c). Likewise, the observed enrichment of \(^{15}\text{N}\) in the \(\text{NO}_3^-\) pool declined with increasing rate from the control treatment over the litter mix treatment to the hemiparasitic litter treatment (Figure 4.5d-f).
4.3.2 Soil N transformation rates

**Rhin-D soil**

All estimated N transformation rates were statistically different between the treatments. Both the gross mineralization of the organic N pool to the NH$_4^+$ pool ($M_{\text{Norg}}$) and the gross immobilization of NH$_4^+$ back to organic N ($I_{\text{NH4}}$) were highest in the control soil, about 35% smaller in the litter mix treatment and about 80% smaller in the hemiparasitic litter treatment compared to the control soil (Figure 4.3a-c, Table 4.2). In contrast, the net N flux from organic N to NH$_4^+$ ($M_{\text{Norg}} - I_{\text{NH4}}$) was an order of magnitude higher in both litter addition treatments compared to the control soil, being 61% higher in the hemiparasitic litter treatment compared to the litter mix treatment. Also the oxidation of NH$_4^+$ to NO$_3^-$ ($O_{\text{NH4}}$) was higher in both litter addition treatments compared to the control soil.

| Table 4.2 Gross N transformation rates (mean and SD) estimated by the tracing model for the three treatments for both soils. Rhin-D: soil from site ‘Doode Bemde’ with litter from *Rhinanthus* and its co-occurring, non-parasitic species; Pedi-H: soil from site ‘Hooiput’ with litter from *Pedicularis* and its co-occurring, non-parasitic species. Fluxes are mineralization of organic N to ammonium ($M_{\text{Norg}}$), immobilization of ammonium to organic N ($I_{\text{NH4}}$), oxidation of ammonium to nitrate ($O_{\text{NH4}}$), oxidation of organic N to nitrate ($O_{\text{Norg}}$), immobilization of nitrate to organic N ($I_{\text{NO3}}$), fixation of ammonium ($F_{\text{NH4}}$) and release of fixed ammonium ($R_{\text{NH4f}}$). |
|---|---|---|---|
| Abbreviation | Kinetics | N transformation rate (μg N g$^{-1}$ day$^{-1}$) |
| | Control | Non-parasitic litter mix | Hemiparasitic litter |
| | Mean | SD | Mean | SD | Mean | SD |
| **Rhin-D** | | | | | | |
| $M_{\text{Norg}}$ | 0 | 9.7 | 0.9 | 6.36 | 0.38 | 2.18 | 0.1 |
| $I_{\text{NH4}}$ | 1 | 9.66 | 0.48 | 6.05 | 0.38 | 1.68 | 0.06 |
| $O_{\text{NH4}}$ | 0 | 0.1 | 0.01 | 0.3 | 0.02 | 0.54 | 0.01 |
| $O_{\text{Norg}}$ | 0 | 0.98 | 0.09 | 0.02 | 0.02 | <0.01 | <0.01 |
| $I_{\text{NO3}}$ | 1 | 1.02 | 0.1 | 0.4 | 0.02 | 0.47 | 0.01 |
| **Pedi-H** | | | | | | |
| $M_{\text{Norg}}$ | 0 | 2.06 | 0.03 | 2.76 | 0.11 | 3.52 | 0.22 |
| $I_{\text{NH4}}$ | 1 | 1.15 | 0.07 | – | – | – | – |
| $O_{\text{NH4}}$ | 1 | 0.02 | <0.01 | – | – | – | – |
| $O_{\text{Norg}}$ | 0 | 0.16 | 0.02 | 0.16 | 0.03 | 0.23 | 0.01 |
| $I_{\text{NO3}}$ | 1 | 0.18 | 0.02 | 0.16 | 0.03 | 0.22 | 0.01 |
| $F_{\text{NH4}}$ | 1 | 2.23 | 0.13 | 6.51 | 0.33 | 6.96 | 0.58 |
| $R_{\text{NH4f}}$ | 1 | 1.67 | 0.15 | 3.92 | 0.25 | 3.47 | 0.89 |

– denotes transformations not considered in the final model
to the control soil, and was 80% higher in the hemiparasitic litter treatment compared to
the litter mix treatment. Oxidation of organic N direct to NO$_3^-$ ($O_{Nrec}$) occurred in the
control soil, but nearly ceased in the litter addition treatments. Gross immobilization of
NO$_3^-$ to organic N ($I_{NO3}$) was similar to $O_{Nrec}$ in the control treatment and was 60% and
54% smaller in the litter mix treatment and the hemiparasitic litter treatment
respectively. All three treatments had a net flux from NO$_3^-$ to organic N ($I_{NO3} - O_{Nrec} >$
zero), but this N flux was an order of magnitude higher in the litter addition treatments
compared to the control soil. For the hemiparasitic litter treatment, the net flux from
NO$_3^-$ to organic N was 24% higher compared to the litter mix treatment.

**Pedi-H soil**

All estimated transformation rates were statistically different between the treatments,
except for $O_{Nrec}$ and $I_{NO3}$ that did not differ between the control soil and the litter mix
treatment. The gross mineralization of organic N to NH$_4^+$ ($M_{Norg}$) increased from control
soil over the litter mix treatment (+34%) to the hemiparasitic litter treatment (+71%)
(Figure 4.3d-f and Table 4.2). Gross immobilization of NH$_4^+$ back to organic N ($I_{NH4}$) only
occurred in the control soil. Therefore, the net flux from organic N to NH$_4^+$ in the litter
addition treatments was substantially higher than in the control soil (+203% for the litter
mix treatment and +287% for the hemiparasitic litter treatment). In the hemiparasitic
litter treatment, this net flux was 28% higher compared to the litter mix treatment.
Oxidation of NH$_4^+$ to NO$_3^-$ ($O_{NH4}$) was negligible in all three treatments. In both litter
addition treatments, the model fit still increased when excluding $O_{NH4}$ and resulted in a
more likely model according to the Akaike’s weights (Table 4.3). The fixation or
immobilization of extractable NH$_4^+$ to fixed NH$_4^+$ ($F_{NH4}$) and the release of fixed NH$_4^+$
($R_{NH4}$) were higher in the litter addition treatments than in the control soil. The net NH$_4^+$
fixation ($F_{NH4} - R_{NH4}$) was about five times higher in the litter addition treatments, being 35% higher in the hemiparasitic litter treatment compared to the litter mix treatment.

The feasibility of the inclusion of the fixed NH$_4^+$ pool (NH$_4^+_{fix}$) was shown by the much higher calculated probability of the model based on Akaike’s weights (Table 4.3).

Direct oxidation of organic N to NO$_3^-$ ($O_{Nrec}$) and gross NO$_3^-$ immobilization to organic N ($I_{NO3}$) were similarly small in all treatments, with a negligible net flux from NO$_3^-$ to organic N ($I_{NO3} - O_{Nrec}$).

**Table 4.3** Value of the Akaike Information Criterion (AIC) and probability (%) of three model setups simulating gross N transformations in the Pedi-H treatments. NH$_4^+_{fix}$ = fixed NH$_4^+$ pool; $O_{NH4}$ = oxidation from NH$_4^+$ to NO$_3^-$; $I_{NH4}$ = immobilization of NH$_4^+$

<table>
<thead>
<tr>
<th>Model (Pedi-H soil)</th>
<th>Control</th>
<th>Non-parasitic litter mix</th>
<th>Hemiparasitic litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC</td>
<td>$p$ (%)</td>
<td>AIC</td>
<td>$p$ (%)</td>
</tr>
<tr>
<td>Exclusion of the NH$<em>4^+</em>{fix}$ pool</td>
<td>1437</td>
<td>&lt;&lt;0.01</td>
<td>4386</td>
</tr>
<tr>
<td>Full model</td>
<td>1015</td>
<td>100</td>
<td>4087</td>
</tr>
<tr>
<td>Full model ($O_{NH4} = 0; I_{NH4} = 0$)</td>
<td>1404</td>
<td>&lt;&lt;0.01</td>
<td>3788</td>
</tr>
</tbody>
</table>

Gross N transformation rates
Figure 4.4 Measured (mean ± SE) and modeled (dashed lines) content of $^{15}$NH$_4^+$ (a-c) and $^{15}$NO$_3^-$ (d-f) as a function of time after $^{15}$N label addition in the three Rhin-D treatments: control soil (a, d), soil amended with a litter mix (b, e) and soil amended with *Rhinanthus* litter (c, f). Small figures (a-c) show the $^{15}$NO$_3^-$ content after addition of $^{15}$NH$_4^+$.
Figure 4.5 Measured (mean ± SE) and modeled (dashed lines) content of $^{15}$NH$_4^+$ (a-c) and $^{15}$NO$_3^-$ (d-f) as a function of time after $^{15}$N label addition in the three Pedi-H treatments: control soil (a, d), soil amended with a litter mix (b, e) and soil amended with *Pedicularis* litter (c, f)
4.4 Discussion

4.4.1 Two contrasting soil types

In a conceptual N model proposed by Schimel and Bennett (2004), the dominant available N form shifts from organic N monomers over NH$_4^+$ to NO$_3^-$ as N availability increases. According to this model, the oligotrophic Pedi-H soil is situated in the zone with moderately low N supply with NH$_4^+$ being the most important DIN form and nitrification being negligible (Schimel and Bennett 2004). On the other hand, the mesotrophic Rhin-D soil is rather situated in the moderately high zone of N supply, where NH$_4^+$ is still the most important DIN form (looking at gross production rates), but also some nitrification occurs.

The gross mineralization rate ($M_{Norg}$) in the mesotrophic Rhin-D soil (9.7 μg g$^{-1}$ soil, Table 4.2, Figure 4.3a) is moderately high compared to those generally observed for grassland soils: while measured values in isotope dilution studies range from 0.3 to 44 μg g$^{-1}$, 86% is below 10 μg g$^{-1}$ soil (Booth et al. 2005). Nearly all mineralized NH$_4^+$ is immobilized again in microbial biomass ($I_{NH4}$), resulting in a small net flux from organic N to NH$_4^+$. The (autotrophic) nitrification rate ($O_{NH4}$) in the Rhin-D soil (0.1 μg g$^{-1}$ soil, Table 4.2, Figure 4.3a) is very small compared to the 0.1-7 μg g$^{-1}$ range observed in other isotope dilution studies for grassland soils (Booth et al. 2005). However, as the $^{15}$N traced in the NO$_3^-$ pool after $^{15}$NH$_4^+$ addition was not modelled well (Figure 4.4a, small figure), the nitrification rate might be underestimated here. As modelled, 91% of NO$_3^-$ is produced by direct oxidation of organic N ($O_{Norg}$). Microbial immobilization of NO$_3^-$ ($I_{NO3}$) is lower than NO$_3^-$ production ($O_{Norg} + O_{NH4}$). As a result, the NO$_3^-$ pool increased throughout the experiment (+0.42 μg g$^{-1}$ soil d$^{-1}$). This build-up of DIN in the soil solution likely indicates
that microbial growth is C limited: microbes use C from dissolved organic matter (DOM) to support their energy needs and release mineral N (Paine 1969; Schimel and Bennett 2004). The relatively low overall C:N ratio of the Rhin-D soil (11:1) – similar to the 8:1 to 12:1 range typical for soil micro-organisms (Wright and Coleman 2000) – at least indicates that C is not plentiful available in the soil.

In contrast to the mesotrophic Rhin-D soil, the gross mineralization rate in the oligotrophic Pedi-H soil (2.06 μg g⁻¹ soil, Table 4.2, Figure 4.3d) is in the lower range of other studies (Booth et al. 2005). As only 56% is immobilized again in microbial biomass, the net flux from organic N to NH₄⁺ is much higher compared to the Rhin-D soil. Nearly no (autotrophic) nitrification (O_NH₄) occurs in the Pedi-H soil (0.02 μg g⁻¹ soil, Table 4.2, Figure 4.3d), likely due to the high soil acidity (pH 4.59): below a pH of 4.5, nitrification becomes negligible (Paine 1969). In addition, according to the conceptual N model of Schimel and Bennett (2004), low autotrophic nitrification is expected in this oligotrophic sandy soil. Instead, NH₄⁺ is rapidly fixed or immobilized to the NH₄⁺_fix pool (and released again), with a concurrent net fixation. Whereas clay minerals such as vermiculite and illite are known to fix NH₄⁺ between their layers (e.g., Röing et al. 2006), NH₄⁺ fixation by clay minerals is not likely in this sandy soil. Another explanation for rapid fixation (or immobilization) of NH₄⁺ in the Pedi-H soil is its high organic matter content (96 ± 16 mg C g⁻¹). Russow et al. (2008) found that an increase in soil organic matter content (SOM) resulted in an increase in strongly adsorbed NH₄⁺ that could not be extracted with 1M KCl. The rapid NH₄⁺ fixation in the Pedi-H soil could further be related to the high C:N ratio (24 ± 1) of the SOM, suggesting a high polyphenolics:N ratio (e.g., Hattenschwiler and Vitousek 2000) typically found in acid dwarf-shrub rich vegetations similar to that of
the Pedi-H site (Eskelinen et al. 2009). Like $O_{\text{NH}4}$, the other N fluxes related to NO$_3^-$ ($O_{\text{Norg}}, I_{\text{NO}3}$) are small as well, with concurrent negligible net NO$_3^-$ immobilization. Throughout the experiment, the NH$_4^+$ pool increased by a similar rate (0.43 $\mu$g g$^{-1}$ soil d$^{-1}$) as NO$_3^-$ did in the Rhin-D soil. Again, this build-up of DIN in the soil solution might indicate that microbial growth is C limited in the absence of a source of labile carbon (Paine 1969; Schimel and Bennett 2004). This is not supported by the C:N ratio of the Pedi-H soil (24:1), which is considerably higher than the 8:1 to 12:1 range of soil microbes (Wright and Coleman 2000). Yet, with the majority of the C presumably bound in recalcitrant organic matter (high C:N), the overall soil C:N might be considerably higher than that of the more labile C compounds used as substrate by micro-organism. Alternatively, the net mineralization might be explained by the high N:P ratio in the soil (20:1, Table 4.1) relative to microbial stoichiometry (5:1) (Cleveland and Liptzin 2007). Therefore, microbial growth is likely to be strongly limited by P in the Pedi-H soil resulting in release of redundant N.

### 4.4.2 Effects of Rhinanthus litter on soil N dynamics

In contrast to expectations, litter addition decreased gross mineralization and NH$_4^+$ immobilization rates (Table 4.2, Figure 4.3a-c). We should be cautious interpreting these results. Like many other (analytical) methods, the gross N fluxes estimated by the model only give a ‘best estimation’. It is possible that the gross mineralization and immobilization fluxes in the litter addition treatments are underestimated. For example, the model fit for dilution of $^{15}$NH$_4^+$ shows a too slow dilution for the hemiparasitic litter treatment (Figure 4.4c). Since the average NH$_4^+$ pool sizes and $^{15}$N enrichment and dilution rate are similar for all three treatments (Figure 4.3a-c, Figure 4.4a-c), we would
expect similar gross mineralization ($M_{\text{Norg}}$) and immobilization rates ($I_{\text{NH}_4}$). The model has been developed for old grassland soils, yet litter addition is a new element not previously tested. When litter is added, gross mineralization ($M_{\text{Norg}}$) might no longer follow zero order kinetics. Future work could try to implement new kinetics to incorporate litter decomposition dynamics.

This being said, a possible explanation for the lower gross mineralization rates when litter is added, can be found in the discrepancy in C:N ratio between the soil and the added litter (Table 4.1). The soil has a C:N ratio (11:1) within the range 8:1 to 12:1 typical for soil microbes (Wright and Coleman 2000); therefore, microbes can easily meet their needs in the control soil. When (hemiparasitic) litter with an increased C:N ratio – and thus C availability – is added to the soil, microbes may become strongly N limited. Therefore, microorganisms will increase their N use efficiency in order to enable microbial homeostasis (i.e., greater microbial investments in N acquisition enzymes for N assimilation, reduced microbial investment in N respiration) (Griffiths et al. 2012; Mooshammer et al. 2012). Concurrently, they may excrete redundant C by decreasing their C use efficiency (overflow mechanism, Manzoni and Porporato 2009). The N is thus, in the short term, tied up into the microbial biomass, leading to reduced gross N mineralization and immobilization rates. In the long term, a further increase in microbial biomass as a result of litter addition will likely result in enhanced microbial turnover, generating labile N inputs that might increase N bioavailability. The differences between *Rhinanthus* litter and the non-parasitic litter mix may be attributed to the quality of the litter; if the higher C:N ratio in the litter mix is related to a higher content of secondary
compound such as polyphenols and lignin, C might actually be less bioavailable compared to the hemiparasitic litter (Quested et al. 2003a; Cornwell et al. 2008).

In contrast to expectations based on stoichiometry (Mooshammer et al. 2012), litter addition with a relatively high C:N ratio (compared to soil C:N) increased (autotrophic) nitrification. This increased nitrification is accompanied by a tenfold increase of the net flux from organic N to NH$_4^+$ and the net immobilization of NO$_3^-$, leading to an increased cycling from organic N over NH$_4^+$ and NO$_3^-$ back to organic N. This increase in N cycling is higher in the hemiparasitic litter treatment compared to the non-parasitic litter mix. We suggest that hemiparasitic litter increases N cycling more than the non-parasitic litter mix as a result of its higher decomposability leading to higher microbial activity. Hemiparasitic litter was indeed shown to decompose faster than litter from many – but not all – co-occurring species in sub-arctic and alpine habitats (Quested et al. 2003a; Spasojevic and Suding 2011). In a chapter 2, we found that *Rhinanthus* leaf litter decomposed extremely fast – losing 93% of its mass in eight months. Litter mass loss in a similar period (nine months) of graminoids (50%) and non-parasitic forbs (63%) at the same sites was considerably smaller (Ameloot et al. unpublished).

The decrease in microbial mineralization-immobilization turnover (MIT) in the hemiparasitic litter treatment compared to the litter mix treatment is more profound than the concurrent increase in N$_{\text{org}}$ - NH$_4^+$ - NO$_3^-$ - N$_{\text{org}}$ cycling (Figure 4.3b-c). Therefore, the potentially plant-available N is lower in the hemiparasitic litter treatment, at least if we trust the modeled microbial MIT (see discussion at the start of 4.4.2) and if plants are able to compete for the rapid microbial MIT of N. However, the microbial MIT turnover measured by pool dilution experiments may reflect microbial cycling and recycling of
Gross N transformation rates

small pools of highly labile, N-rich compounds rather than the overall breakdown of soil organic matter (Fierer et al. 2001). Since it is the latter that regulates overall N cycling (Schimel and Bennett 2004), microbial MIT may be a poor predictor of N availability to plants. In the case of litter addition, net mineralization ($M_{Nec} - l_{NH4} - O_{NH4}$) is not a good predictor either, as it is highly dependent on the particular moment of measurement – often with initial net immobilization changing in time to net mineralization (Manzoni et al. 2008). For these reasons, we propose that the $N_{org} - NH_4^+ - NO_3^- - N_{org}$ cycling (Figure 4.3b-c) – increasing from control over non-parasitic litter to Rhinanthus litter – to represent best the relative differences in plant-available nitrogen between treatments.

The increased turnover of NO$_3^-$ by hemiparasitic litter in particular might be important for plant uptake, as plants compete more effectively with microbes for the more mobile NO$_3^-$, in contrast to NH$_4^+$ (Recous et al. 1988). Together, our results suggest that considerably more N is available for plant uptake in soil with Rhinanthus litter compared to soil with the litter mix. Moreover, the higher NO$_3^-$ immobilization ($I_{NO3}$) in the hemiparasitic litter treatment can enhance N supply in the long term by avoiding N losses (leakage, denitrification), and ultimately increases the active organic N pool (Recous et al. 1988). Nitrate consumption ($I_{NO3}$) includes, in addition to NO$_3^-$ immobilization, also potential denitrification and leaching losses. Since no leaching could occur from the plastic containers and denitrification only occurs in anaerobic microsites (Araujo et al. 2005), practically all NO$_3^-$ consumption in the present study can be attributed to immobilization. Where NO$_3^-$ immobilization was in the past commonly thought to be minimal (Tiedje et al. 1981; Myrold and Tiedje 1986), nitrate immobilization rates approaching gross nitrification rates have been reported for

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agricultural, grassland and forest soils (Stark and Hart 1997; Hatch et al. 2000; Burger and Jackson 2003).

4.4.3 Effects of *Pedicularis* litter on soil N dynamics

Litter addition increased both gross and net mineralization rates compared to the control soil (Table 4.2, Figure 4.3d-f). Since the immobilization to organic N ($I_{NH_4}$) equaled zero in the litter amended soils, the net flux from organic N to NH$_4^+$ equaled the gross mineralization rate. The mineralization rate was 28% higher in the hemiparasitic litter treatment compared to the litter mix treatment. 93% and 99% of mineralized N ended up in the fixed NH$_4^+$ pool in the litter mix and hemiparasitic litter treatment respectively. The fact that no gross immobilization was modeled explicitly ($I_{NH_4} = 0$), does not mean that no immobilization of NH$_4^+$ occurred; more likely, microbial immobilization is now included in the NH$_4^+$ fixation rate ($F_{NH_4}$). This makes sense as similar NH$_4^+$ fixation rates were expected across all three treatment (using the same soil), because fixation was thought to occur primarily to SOM and less so to the litter. Alternatively, NH$_4^+$ fixation rates could increase with standing NH$_4^+$ pool (typical for first order kinetics). In contrast, gross and net NH$_4^+$ fixation rates were higher at lower NH$_4^+$ levels. While it is possible that some of the NH$_4^+$ is fixed to the added litter, it is more likely that the fixed NH$_4^+$ pool (NH$_4^+_{fix}$) also includes rapid microbial MIT related to microbial cellular processes.

The potentially plant-available N (the gross production of NH$_4^+$ as NO$_3^-$ turnover is negligible) is the sum of the fluxes from organic N and NH$_4^+_{fix}$ to NH$_4^+$ ($M_{org} + R_{NH_4}$). This gross production of NH$_4^+$ is significantly higher (6.99 ± 0.10) for the *Pedicularis* litter treatment compared to the non-parasitic litter mix treatment (6.68 ± 0.08). However, as
discussed above for *Rhinanthus*, the fast microbial MIT is related to microbial cellular processes rather than the overall breakdown of organic N and therefore not a good predictor of plant-available N (Fierer et al. 2001; Schimel and Bennett 2004). As the turnover of the NH$_4^+$$_{\text{fix}}$ in the litter treatments is likely to include microbial MIT in addition to abiotic fixation-release processes, we suggest it is better to consider the $N_{\text{org}}$-NH$_4^+$-NH$_4^+$$_{\text{fix}}$ turnover – increasing from control soil over the non-parasitic litter mix treatment to the hemiparasitic litter treatment – as an indicator for the effect on plant-available N. We can think of it as a net transition from a more recalcitrant organic N pool ($N_{\text{org}}$) to a highly labile organic and fixed N pool (NH$_4^+$$_{\text{fix}}$). Therefore, N will likely be more available to plants, at least in the long term. Together, our results suggest that N is more available for plant uptake in soil with *Pedicularis* litter compared to soil with the litter mix; however, the relative effect is less pronounced than for *Rhinanthus* litter.

**4.4.4 The litter pathway confirmed**

To the best or our knowledge, this is the first detailed report on the impact of hemiparasitic litter on the soil N cycle. We compared the effects on gross soil N transformations between the litter of two hemiparasitic plants and a litter mix of co-occurring non-parasitic species from the same communities. In general, adding hemiparasitic litter increased N fluxes between the different N pools more than adding the litter mix of co-occurring species (Figure 4.3): from organic N over NH$_4^+$ and NO$_3^-$ back to organic N in the Rhin-D soil and from organic N over NH$_4^+$ to fixed or immobilized NH$_4^+$$_{\text{fix}}$ in the Pedi-H soil. This is also reflected by the increasing rate of dilution of NO$_3^-$ in the Rhin-D soil and of NH$_4^+$ in the Pedi-H soil from control over litter mix to hemiparasitic litter treatment (Figure 4.4 and Figure 4.5). As discussed under
point 4.4.2 and 4.4.3, the lack of litter decomposition dynamics in the zero order implementation of the mineralization flux ($M_{\text{org}}$) and the hard-to-define fixed NH$_4^+$ pool (NH$_4^+_{\text{fix}}$) formed potential pitfall in the model interpretation. To improve the credibility of the results, further research could make the model more appropriate for litter addition experiments and experiment more with the NH$_4^+_{\text{fix}}$ pool.

Our interpretation of the present study supports the existence of a litter pathway in which hemiparasitic plants enhance nutrient availabilities through the production of high-quality litter with possible effects on community structure and species diversity (Spasojevic and Suding 2011). Yet, explaining gross soil N dynamics and its translation to N availability to plants in the field is not straightforward. To confirm these results, future experiments could add $^{15}$N labeled hemiparasitic litter to field plots and trace the subsequent $^{15}$N uptake by the vegetation (chapter 5). The effect of *Pedicularis* litter on net N transformation rates was relatively small compared to the effect of *Rhinanthus* litter: the net flux from organic N to NH$_4^+$ increased by 61% when *Rhinanthus* litter was added and by 28% when *Pedicularis* litter was added relative to addition of a non-parasitic litter mix. This contrasts with the expectation that litter effects of hemiparasitic plants on N availability would be more important in oligotrophic sites (*Pedicularis*) compared to mesotrophic sites (*Rhinanthus*), but note that the soil types is confounded with the litter types. Research on the mistletoe *Amyema miquelii* revealed that its impact on annual litter P and potassium (K) returns were substantial more important than on N returns (March and Watson 2010). The relatively low N:P ratio of *Pedicularis* litter compared to the Pedi-H soil and non-parasitic litter suggests that effects of *Pedicularis* litter on P availability will be relatively higher than on N availability. In
particular because the N:P ratio of the soil (20:1) is substantially higher than microbial requirements (5:1), suggesting P limitation (Cleveland and Liptzin 2007). In contrast, the N:P ratio of *Rhinanthus* litter is similar to the soil and to non-parasitic litter. Future research on the effects of hemiparasitic litter should therefore also focus on other nutrients such as P and K.
Vegetation N uptake from litter of *Rhinanthus* and *Pedicularis*


Abstract

Hemiparasitic plants often produce nutrient-rich litter with high decomposition rates, and thus can enhance nutrient availability. When plant species have differential affinities for this nutrient source, hemiparasitic litter might influence species composition in addition to the parasitic suppression of host species. We expected that species adapted to fertile habitats derive a higher proportion of nutrients from the hemiparasitic litter compared to other species. $^{15}$N-labelled litter of *Rhinanthus angustifolius* and *Pedicularis sylvatica* was added to experimental field plots and adjacent litter bags. We examined N release from the litter, N uptake by the vegetation 2, 4 and 12 months after litter addition and differences in the proportion of N taken up from the litter ($N_L$) between co-occurring species. The percentage of N in shoots of co-occurring plant species that is derived from the added hemiparasitic litter ($N_L$) strongly differed between the species (0.1-6.2%). After exclusion of species with an alternative N source (legumes as well as ectomycorrhizal and ericoid mycorrhizal species), $N_L$ was positively related ($p <$
0.001) with specific leaf area (SLA) and at *Pedicularis* sites with leaf N concentration (LNC) and leaf phosphorus concentration (LPC) \( p < 0.05 \), i.e., leaf traits associated with a fast-growth strategy and adaptation to high-nutrient environments. Our results suggest that nutrient release from hemiparasitic litter favors plant species with a fast-growth strategy adapted to high-nutrient environments compared to species with a slow-growth strategy. Whether continued hemiparasitic litter inputs are able to change species composition in the long term requires further research.

### 5.1 Introduction

Hemiparasitic plants form haustorial connections to host species through which they take up water, nutrients and carbon compounds, resulting in performance reduction of the host species (Kuijt 1969; Pate 1995; Press 1995). If certain hosts are preferred over others, parasitism can alter the competitive relations between co-occurring species (Gibson and Watkinson 1991; Matthies 1996; Press et al. 1999). For instance, the decrease of total, graminoid and legume biomass in grasslands infected with *Rhinanthus* spp. is thought to alter the species composition in favor of non-leguminous forbs and to increase local plant diversity (Gibson and Watkinson 1991; Davies et al. 1997; Ameloot et al. 2005).

On the other hand, hemiparasitic plants accumulate nutrients in their tissues, which is thought, in part, to be a result of their high transpiration rates (Gauslaa 1990; Gauslaa and Odasz 1990; Pate 1995; Phoenix and Press 2005) and therefore produce litter with high decomposability (Seel and Press 1993; Press 1998; Press et al. 1999; Quested et al. 2002; Quested et al. 2003a). While hemiparasitic litter feedbacks on species composition remain unstudied, several studies reported positive effects of hemiparasitic presence or their litter on N cycling: Quested et al. (2003a; 2003b) found a 42 % increase of total
Vegetation N uptake from litter

annual nitrogen (N) input to the soil in the vicinity of the hemiparasite *Bartsia alpina* as well as a corresponding increase in plant growth for species grown with *B. alpina* litter compared to litter of co-occurring species; in a mesocosm study, Bardgett et al. (2006) reported higher N mineralization rates in pots with *Rhinanthus minor* compared to pots without; in a $^{15}$N tracing experiment, Ameloot et al. (2008) observed that the added $^{15}$NH$_4$$^{15}$NO$_3$ (ammonium nitrate) was more diluted in plots parasitized with *Rhinanthus* spp. compared to control plots, suggesting larger soil N pools in parasitized plots; March and Watson (2010) found that mistletoe infection increased litter N returns by 65% because of the high rate of leaf litterfall. The increase in nutrient availability due to hemiparasitic litter inputs might compensate for the biomass loss due to parasitism, resulting in a net biomass increase. For example, Spasojevic and Suding (2011) reported that the aboveground biomass in alpine tundra was two-fold higher in plots with the hemiparasite *Castilleja occidentalis* compared to plots without. Similar to nutrient addition experiments in temperate grasslands, which showed an increase in graminoid biomass with a consequent diversity loss of mainly small perennial grasses and forbs (Silvertown et al. 2006; Hejcman et al. 2007; Hautier et al. 2009; De Schrijver et al. 2011), hemiparasitic litter feedbacks could reduce local species diversity. However, Spasojevic and Suding (2011) found only a weak effect on species abundance and no effect on diversity or species composition.

In this chapter, we identified which species are favored by hemiparasitic litter feedbacks. We therefore added $^{15}$N-labeled litter of two short-lived hemiparasitic plant species to field plots and quantified how much was taken up by individual plant species. We focused on N as a proxy for the biogeochemical effects of hemiparasitic litter because it is easy to trace using the stable isotope $^{15}$N. The litter produced when short-lived
hemiparasites die off can be considered as a pulse supply of nutrients where plant competition is about successfully pre-empting this supply from neighboring plants (Craine 2005). Following a pulse supply of nutrients, plants with a rapid increase in root length of sufficient uptake capacity are expected to win the race. Root traits are not easy to determine and have not been measured for a wide range of species. However, root uptake capacity was found to be related to specific leaf area (SLA, leaf area per mass) (Osone et al. 2008), which is available for many more species. Among others, SLA and mass-based leaf N and phosphorus (P) concentrations (LNC and LPC) are closely correlated and together represent the ‘leaf economics spectrum’, ordering plants from slow to quick returns on investments and growth on a global scale (Wright et al. 2004). These leaf economy traits also represent the competition-stress tolerance (C-S) axis of the well-established CSR scheme (Grime 1977; Westoby 1998), with SLA decreasing from along the C-S axis. Moreover, SLA, LNC and LNP across species and sites from all continents have been reported to increase with soil fertility (Ordonez et al. 2009). Thus, high-nutrient environments seem to select for species with a fast-growth strategy, though there is considerable variation in plant strategies within the same site (Westoby et al. 2002). In addition to leaf traits, Ellenberg N values represent realized ecological niches of species along a fertility gradient for Central Europe (Ellenberg and Leuschner 2010).

We hypothesized, in line with nutrient addition experiments, that graminoids are better competitors for nutrients than other growth forms and thus have higher contents of total shoot N derived from added litter \( (N_L) \). Looking at leaf traits, we expected \( N_L \) values to increase with SLA, LNC and LPC, as these are characteristic for a fast-growth strategy and associated with a high nutrient supply. Finally, we expected species with higher
Ellenberg N values, which are adapted to more productive environments, to have higher $N_L$ values. These hypotheses were tested for 51 species using litter from two contrasting hemiparasitic plant species: *Rhinanthus angustifolius* C.C. Gmel. and *Pedicularis sylvatica* L., hereafter referred to as *Rhinanthus* and *Pedicularis*. *Rhinanthus* typically grows in mesotrophic grasslands belonging to the *Molinio-Arrhenatheretea* class (*sensu* Zuidhoff et al. 1996), while *Pedicularis* thrives in relatively oligotrophic heath-grasslands belonging to the *Nardetea* class (*sensu* Swertz et al. 1996). Both species are early flowering, short-lived root hemiparasites belonging to the cosmopolitan *Orobanchaceae* family.

### 5.2 Materials and methods

This study was carried out in the same three mesotrophic *Rhinanthus* sites and three oligotrophic *Pedicularis* sites described in chapter 2 (part 2.2.1, Table 2.1). While *Rhinanthus* and, most likely, *Pedicularis* are not host-specific (Weber 1976; Kuijt 1979), host preference has been reported for both *Rhinanthus* and *Pedicularis* species (e.g., Gibson and Watkinson 1991; Ren et al. 2010).

#### 5.2.1 Production of labelled hemiparasitic litter

At every site, we selected a field plot of 1 m² with abundant hemiparasite cover. Each plot was sprayed twice with a solution of $^{15}$NH$_4$Cl (886 mg L$^{-1}$) using an electrical backpack sprayer (0.82 L min$^{-1}$): a first time on 22-Apr-2010, after emergence of both hemiparasites, and a second time on 21-May-2010, before flowering. The sprinkler head was moved at a constant rate in crossed strips assuring a homogeneous distribution of the label solution. Sprinkling times were chosen to achieve similar $^{15}$N enrichment in both litter types, based on pre-test: 1 min m$^{-2}$ for *Pedicularis* and 2 min m$^{-2}$ for
Rhinanthus. Plants were collected on 14-Jun-2010 and air-dried in the laboratory. Since the management is inherently connected to the presence of the hemiparasites, only the part of the plant that returns to the soil as litter under the management regime was used in our experiment: stems of Rhinanthus were removed as they are removed by mowing, while entire Pedicularis plants were used as only the tips are removed by mowing adjusting the applied amounts for the tip removal.

5.2.2 Field experiment

On all six sites we selected six 50x50 cm plots. Three of the six plots served as control plots for $^{15}$N natural abundance measurements, while in the other three plots $^{15}$N labeled hemiparasitic litter was added. The litter addition plots were separated from the control plots by a 0.5 m buffer zone to avoid contamination of the control plots (Figure 5.1). Before litter addition on 1-Jul-2010, all shoot biomass was clipped and removed. Shoot removal allowed us to analyze only new growth, meaning that the analyzed shoot biomass was produced in the presence of the labeled litter. The shoot removal also simulates the mowing management in these semi-natural grasslands. Added hemiparasitic litter quantities (Table 5.1) are site-specific and realistic for vegetation patches with high hemiparasite abundance (based on the densities calculated from the 1 m² litter collection plots). The added N amount (1.2-2.2 g hemiparasitic litter N m⁻²) is in the lower range of that in nutrient addition experiments (0.5-48 g N m⁻² year⁻¹) in the

<table>
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<th>Added litter</th>
<th>Mesotrophic Rhinanthus sites</th>
<th>Oligotrophic Pedicularis sites</th>
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</thead>
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<tr>
<td></td>
<td>Rhin-A</td>
<td>Rhin-D</td>
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<tr>
<td>DW 25°C (g m⁻²)</td>
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<td></td>
</tr>
<tr>
<td>60</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>N (g m⁻²)</td>
<td>1.68</td>
<td>1.83</td>
</tr>
</tbody>
</table>
Vegetation N uptake from litter

(meta-analysis of De Schrijver et al. 2011). A wooden frame (10 cm belowground and 10 cm aboveground) was installed around the plots to protect the added litter from being blown away and to avoid $^{15}$N uptake by plants outside the plot. At each site, one out of three control and treated plots were sampled after 2, 4 and 12 months after litter addition. Sampling encompassed the clipping and sorting per species of the total shoot biomass at 1 cm above ground level and pooled sampling of subplots for the ‘moss-litter’ layer (subplot diameter (d) = 11.4 cm; n = 3 per plot) and soil layers of 0-5 cm depth (d = 5.0 cm; n = 3 per plot) and 5-15 cm (d = 4.25 cm; n = 3 per plot). The three subplots were pooled prior to chemical analysis. The ‘moss-litter’ layer is the remaining fraction of loose organic material left over after the clipping of aboveground biomass and consists mainly of mosses and litter (including the residues of the added hemiparasitic litter).

Shoot biomass of the plots that are sampled after 1 year was also clipped and removed intermediately after 4 months to represent the mowing management.

Figure 5.1 Photographical representation of the experimental setup per site. One control plot and one litter addition plot were harvested after 2 (t1), 4 (t2) and 12 (t3) months. The wooden frame prevents the litter from being blown away.
Chapter 5

In order to monitor the N release rate from the applied hemiparasitic litter, 20 x 20 cm² litterbags (mesh size 1.5 mm, n = 2 per sampling date) filled with 1.5 g air-dried (25°C) *Rhinanthus* litter or 3 g *Pedicularis* litter were put in close contact with the ground. Two litter bags were removed for analysis at each of the three sampling times (2, 4 and 12 months after installation).

### 5.2.3 Chemical analyses

Litter samples were first air-dried (25°C) and weighed to determine mass loss. All shoot and litter samples were dried at 70°C for 48 h and weighed. Samples < 100 mg were excluded from further analysis as insufficient sample remains for analysis after grinding. The remaining samples were ground with an ultra centrifugal mill (mesh size 0.5 mm) (ZM200, Retsch, Germany). Soil samples were dried at 40°C for 96 h, and after removal of roots, soil samples were ground with a planetary ball mill (PM400, Retsch, Germany). Subsamples were analyzed for total N and $^{15}$N using an elemental analyzer (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (20-20, SerCon, UK). Cross-contamination was avoided by including method blanks and analytical quality was checked by the standard deviation of two replicated analyses per sample.

### 5.2.4 Calculations

The cumulative N release from the added hemiparasitic litter ($N_{\text{released}}$, g N m$^{-2}$) was calculated accounting for the difference in $^{15}$N enrichment between litter addition and sampling time using the litterbag data:

$$N_{\text{released}}(g \text{Nm}^{-2}) = \frac{N_{\text{litter,t}} - a_{\text{litter,t}} - N_{\text{litter,0}}}{a_{\text{litter,0}}}$$  \hspace{1cm} (5.1)

$$a^{15} = \frac{^{15}N}{^{15}N + ^{14}N}$$  \hspace{1cm} (5.2)
Vegetation N uptake from litter

with $N_{litter,\,t_0}$ and $N_{litter,\,t}$ the amount of N in the hemiparasitic litter (g m$^{-2}$) at time of addition ($t_0$) and time $t$, respectively; and $^{15}a_{litter,\,t_0}$ and $^{15}a_{litter,\,t}$ the fractional abundance of $^{15}$N in litter at time of addition ($t_0$) and time $t$, respectively. The percentage of shoot N of a species that is derived from the added hemiparasitic litter ($N_L$, %) was calculated using the fractional abundance of $^{15}$N of that species in the litter addition plot ($^{15}a_{treated}$) and the fractional abundance of $^{15}$N of the added litter ($^{15}a_{litter}$), accounting for the natural abundance of $^{15}$N in the species in the control plot ($^{15}a_{control}$), modified from Harrison (2011):

$$N_L(\%) = \frac{^{15}a_{treated} - ^{15}a_{control}}{^{15}a_{litter} - ^{15}a_{control}} \times 100 \quad (5.3)$$

The total aboveground N uptake from the added hemiparasitic litter (g N m$^{-2}$) was calculated as the sum of N uptake, by all individual higher plant species ($\Sigma N_L \cdot N$), with $N$ being the N content (g N m$^{-2}$) of a species.

### 5.2.5 Statistical analysis

For simple comparisons we used Student’s one-sample and two-sample t-tests. Plant traits (LNC, LPC, SLA) were requested from the TRY database (Kattge et al. 2011): 50 datasets provided 4396 records for 51 species (Shipley 1995; Cornelissen 1996; Cornelissen et al. 1996; Atkin et al. 1997; Bahn et al. 1999; Hickler 1999; Medlyn et al. 1999; Meziane and Shipley 1999; Niinemets 2001; Shipley 2002; Cornelissen et al. 2003; Loveys et al. 2003; Ogaya and Penuelas 2003; Quested et al. 2003a; Diaz et al. 2004; Wright et al. 2004; Bakker et al. 2005; Crane et al. 2005; Han et al. 2005; Louault et al. 2005; Bakker et al. 2006; Kerkhoff et al. 2006; Campbell et al. 2007; Garnier et al. 2007; Cornwell et al. 2008; Kleyer et al. 2008; Reich et al. 2008; van Bodegom et al. 2008;
Craine et al. 2009; Kattge et al. 2009; Poorter et al. 2009; Reich et al. 2009; Freschet et al. 2010; Ordonez et al. 2010). We related the aboveground N derived from parasitic litter of all the sampled species (i.e., N_L) to their trait values and Ellenberg N indicator values (Ellenberg and Leuschner 2010) as well as their growth form (graminoids, legumes, non-leguminous forbs, woody plants). While it would be very interesting to test whether N_L differs between host and non-host species, very limited and often conflicting data are available. We applied mixed effects models using the lme4 package in R 2.14.1 (the R Development Core Team 2011). We used the model:

$$\log(N_L) = X + (1|species) + (1|plotID)$$  \hspace{1cm} (5.4)

with N_L the N derived from litter (Equation 5.3), X being the fixed independent variable (e.g., SLA, LNC) and with random intercepts accounting for the non-nested effects of species identity and plotID (unique code for each combination of location and time). The random effects for species were added because most species occurred in several plots, resulting in non-independent trait-combinations and N_L values among the replicates in the model. Models were validated by graphical inspection of normality and homogeneity. A likelihood ratio test was used to compare the model in Equation 5.4 with the intercept-only model (Zuur et al. 2009). Tukey’s honestly significant difference test was used to compare N_L values between growth forms. The whole analysis was done once for all species and once excluding plants associated with N-fixing bacteria (legumes: Lotus uliginosus, Vicia cracca, Lathyrus pratensis), plants with ectomycorrhiza (ECM: Betula spp., Quercus spp. and Salix aurita) and ericoid mycorrhiza (ERM: Calluna vulgaris and Erica tetralix) (Brundrett 1991), as these plant-microbial associations allow access to nutrients not available to other plants: legumes access N from the air, while
ECM and ERM plants access N from organic substances that are not (or less) available to other plants (Brundrett 1991; Read 1991; Aerts and Chapin 2000).

5.3 Results

5.3.1 $^{15}$N recovery

Total $^{15}$N recovery at *Pedicularis* sites was on average 74 ± 9 %, of which 64 ± 8 % was found in the litter layer, and 35 ± 12 % in the 0-15 cm soil. A significant portion of $^{15}$N was not recovered (one-sample $t(8) = 5.04$, $p = 0.001$). Total $^{15}$N recovery at *Rhinanthus* was on average 74 ± 4 %, of which 34 ± 6 % was found in the litter layer, and 63 ± 7 % in the 0-15 cm soil. Also here, a significant portion of $^{15}$N was not recovered (one-sample $t(8) = 4.33$, $p = 0.003$).

5.3.2 N release from litter and uptake by shoots

In the first 2 months (July and August), 32% of *Pedicularis* litter N (one-sample $t(2) = 4.7$, $p = 0.04$) was released and an insignificant 0.5% of the added litter N ended up in shoot biomass ($t(2) = 3.5$, $p = 0.07$) (Figure 5.2). In the same period, 62% of *Rhinanthus* litter N ($t(2) = 10.1$, $p = 0.01$) was released and 2.2% of the added litter N ended up in shoot biomass ($t(2) = 10.9$, $p = 0.008$). During September and October there was no significant N release or uptake from both hemiparasitic litter types. In the subsequent period, from November to June, another 33% of *Pedicularis* litter N ($t(2) = 5.3$, $p = 0.03$) was released and 0.7% ($t(2) = 5.5$, $p = 0.03$) of the added litter N was taken up by new growth of shoots in spring. In the same period, 7.0% of *Rhinanthus* litter N ($t(2) = 4.8$, $p = 0.04$) was released and 1.8% of the added litter N ended up in shoot biomass($t(2) = 10.9$, $p = 0.008$).
Figure 5.2 Mean (± SE) relative (%) N release from the added hemiparasitic litter and subsequent uptake by the vegetation (aboveground) at *Rhinanthus* sites (dashed lines and triangles) and *Pedicularis* litter (solid lines and circles).
Though *Rhinanthus* litter had released roughly double amounts of N compared to *Pedicularis* litter after both two months ($t(2) = 3.3, p = 0.03$) as well as four months ($t(2) = 4.2, p = 0.01$), the cumulative N release from the litterbags after 12 months was similar for both litter types (Figure 5.2; $t(2) = 1.4, p = 0.2$). Yet, the fraction of the added litter N that ended up in the shoots of higher plants after 12 months was nearly four times smaller ($t(2) = 9.9, p < 0.001$) at *Pedicularis* sites (1.06 %) compared to *Rhinanthus* sites (4.15 %).

The average percentage of total shoot N that is derived from the added litter ($N_L$) was highest after 4 months: 0.98 ± 0.03 % at *Rhinanthus* sites and 0.72 ± 0.13 % at *Pedicularis* sites. Twelve months after litter addition, which only includes new spring growth because of shoot removal in autumn, $N_L$ values were about half of those measured 2 and 4 months after litter addition: 0.46 ± 0.04 % for *Rhinanthus* sites and 0.46 ± 0.11 % for *Pedicularis* sites.

### 5.3.3 Interspecific differences in $N_L$

Species differed substantially in their average $N_L$ values, with relatively few species in the high range (Figure 5.3). Hemiparasitic litter N provided up to 5.2% (*Lychnis flos-cuculi* at *Rhinanthus* sites) and 6.2% (*Luzula multiflora* at *Pedicularis* sites) of the shoot N content of individual species in the same year of litter addition (average of 2 and 4 month data). However, values for other species in the same period were as low as 0.17% (*Equisetum palustre* at *Rhinanthus* sites) and 0.25% (*Juncus acuta* at *Pedicularis* sites). $N_L$ values of new growth in the year after litter addition had dropped for nearly all species and this drop was most severe for the few high-ranked species, without changing the ranking much (Figure 5.3). Only at *Rhinanthus* sites, growth form and SLA explained a
Figure 5.3  Species ordered by average $N_l$ (%) values: full circles represent the average of 2 and 4 months after litter addition, open circles are for new growth 12 months after litter addition. Error bars (±SE) are drawn in case of more than one observation. Symbol sizes represent species cover (5-35%, averages of 2010 and 2011). Only species of which at least 2 records of $N_l$ were available are shown; *Betula* spp. = *Betula pendula*/B. *pubescens*; species that occurred in both vegetation types are underlined.
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marginally significant and a significant amount of variation in \( N_L \) respectively (Table 5.2, Figure 5.4). A post-hoc test for growth form revealed that only legumes scored marginally lower \( (z = 2.2, p = 0.07) \) than non-leguminous forbs. Graminoids were not different from forbs \( (z = 0.8, p = 0.8) \) or legumes \( (z = 1.6, p = 0.2) \). When excluding species with an alternative N strategy (legumes, ECM and ERM species), growth form was no longer significant at \textit{Rhinanthus} sites, SLA was significant at both \textit{Rhinanthus} and \textit{Pedicularis} sites, and LNC and LPC explained small but significant amounts of variation in \( N_L \) at \textit{Pedicularis} sites. Ellenberg N-values did not explain any significant part of the variation in \( N_L \) values.

| Table 5.2 | Variation in nitrogen derived from hemiparasitic litter \((N_L)\) between species explained by the traits specific leaf area (SLA), leaf nitrogen concentration (LNC) and leaf phosphorus concentration (LPC), Ellenberg N and growth form (categorical variable, see material and methods). A likelihood ratio (L-ratio) test was used to compare the model in Equation 5.4 with the intercept-only model (Zuur et al. 2009). No results are shown when the model has a higher Akaike information criterion (AIC) than the intercept-only model. Because traits and Ellenberg N values were not available for all species, the total number of observations \((n_{tot})\) and the number of species \((n_{spec})\) are given for each analysis. LEG: Leguminous; ECM: ectomycorrhizal; ERM: ericoid mycorrhizal |
|-----------|-------------------------------------------------|---------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( X \)   | \( n_{tot}/n_{spec} \) | \( \text{L-ratio} \) | \( P \)         | \( n_{tot}/n_{spec} \) | \( \text{L-ratio} \) | \( P \)         |
| \textit{Rhinanthus} sites | | | | | | |
| SLA | 116/34 | 13.17 | <0.001*** | 107/31 | 18.68 | <0.001*** |
| LNC | 96/27 | 3.07 | 0.08 | 87/24 | - | - |
| LPC | 91/24 | - | - | 82/21 | - | - |
| Ellenberg N | 70/22 | - | - | 69/21 | - | - |
| Growth Form | 117/35 | 5.96 | 0.051 | 108/32 | - | - |
| \textit{Pedicularis} sites | | | | | | |
| SLA | 50/18 | - | - | 37/12 | 4.31 | 0.038* |
| LNC | 43/16 | - | - | 30/10 | 5.75 | 0.017* |
| LPC | 40/14 | - | - | 27/8 | 5.24 | 0.022* |
| Ellenberg N | 41/13 | - | - | 36/11 | - | - |
| Growth Form | 51/19 | - | - | 38/13 | - | - |
Figure 5.4 $N_l$ (%) in relation to specific leaf area (SLA), leaf N concentration (LNC) and leaf P concentration (LPC) at Rhinanthus (A) and Pedicularis (B) sites. Symbols represent different growth forms: graminoids (Gramineae, Cyperaceae and Juncaceae, open circles), non-leguminous forbs (open triangles), legumes (Rhinanthus only, filled circles), ECM and ERM species (Pedicularis only, filled squares and triangles). Linear regressions for graminoids and non-leguminous forbs (open symbols) are added only when the leaf trait explained a significant amount of the variation in $N_l$ (Table 5.2).
5.4 Discussion

Nutrient inputs from hemiparasitic litter are thought to increase host and non-host productivity and therewith provides another mechanism – apart from parasitic suppression of host species – by which hemiparasites can influence community structure and diversity. In the two temperate semi-natural grassland types studied here, we found that the overall effect is rather small. Though 90% and 75% of *Rhinanthus* and *Pedicularis* litter N are released within 12, only 1.8% and 0.7% of the added litter N ended up in the total aboveground vegetation. Yet, the considerable interspecific variation in the percentage of N derived from the hemiparasitic litter was related to leaf traits indicative for a fast-growth strategy (Figure 5.4); this suggests that hemiparasitic litter promotes species adapted to high-fertility habitats.

5.4.1 $^{15}$N unaccounted for

At both vegetation types, 26% of the $^{15}$N added as hemiparasitic litter was not recovered in soil (0-15 cm), litter or plant shoots. Besides the possibility that some litter was blown over the 10 cm edge by strong winds, possible losses of $^{15}$N include leaching to deeper soil layers after nitrification to inorganic NO$_3^-$, losses to the atmosphere by denitrification and uptake by deep-rooting plants and fungi from outside the plot. NO$_3^-$ and N$_2$O losses are not expected to be important in these unfertilized meso- and oligotrophic grasslands, where the vegetation N demand is presumably not saturated.

5.4.2 N release and uptake

A considerable proportion of litter N was released within the first two months after litter addition. This confirms our statement that the litter produced when short-lived hemiparasites die off can be considered as a pulse supply of nutrients. Data on chemical
composition from a previous litterbag experiment (Table 2.4 in chapter 2) revealed that the higher lignin:N in *Pedicularis* litter (6.8 ± 0.5) compared to *Rhinanthus* litter (3.2 ± 0.1) was able to explain the difference in decomposition rate between both litter types. The fraction of the added litter N that ended up in the shoots of higher plants after 12 months was nearly four times smaller at *Pedicularis* sites compared to *Rhinanthus* sites, despite the similar N release (roughly 80%). The faster (double) initial N release from *Rhinanthus* litter compared to *Pedicularis* litter can explain some, but not all, of the difference. Different shoot:root allocation ratios provide a second possible explanation.

We found support for this idea by measuring $^{15}$N in roots removed from soil samples (0-15 cm) collected 2 months after litter addition in two sites (Pedi-H and Rhin-D): the shoot:root allocation ratio of the $^{15}$N tracer was 6.1 at Rhin-D, but only 1.6 at Pedi-H. A third possible reason for the smaller N uptake in *Pedicularis* shoots is the incorporation of N in recalcitrant soil organic matter (SOM) with slow mineralization rates, decreasing the N availability to higher plants: the topsoil (0-5 cm) at *Pedicularis* sites has an average C:N value of 24 compared to only 11 at *Rhinanthus* sites (Table 4.1 in chapter 4). Soil C:N ratios are negatively correlated with N mineralization rates and C:N = 15 is a critical value separating soil groups with higher and lower N release (Van Dijk 1968; Springob and Kirchmann 2002; Springob and Kirchmann 2003). A last possible reason is N uptake by bryophytes, which are more abundant at *Pedicularis* sites (personal observation).

The recovery of $^{15}$N in shoot biomass found in other $^{15}$N litter addition experiments—though these were performed in forests and used longer time frames—is within the same range as in the present study (1-4%): Zeller et al. (2000) found that three years after addition of $^{15}$N labelled beech litter, 2% had accumulated in trees; Swanston and
Myrold (1997) estimated a clearcut vegetation $^{15}$N recovery of 2.7% 22 months after addition of labelled alder leaves. In a $^{15}$N addition experiment with mineral N in grasslands similar to our *Rhinanthus* sites, adding 0.7-1 g $^{15}$NH$_4^{15}$NO$_3$-N m$^{-2}$ in early spring resulted in 1.5 to 3 % recovery in shoot biomass after four months (Ameloot et al. 2008); we found a similar average recovery (2.4 %) in shoot biomass at *Rhinanthus* sites after four months. Since the N from both hemiparasitic litter types provided similar fractions of total shoot N ($N_L$ values), the higher recovery of *Rhinanthus* litter N in shoot biomass can be attributed to the higher productivity in these sites. The cumulative shoot production over the year was on average 2.8 times higher at *Rhinanthus* sites compared to *Pedicularis* sites.

### 5.4.3 Explaining interspecific differences in $N_L$

Following a pulse-supply of N, we expected graminoids to be favored more than other growth forms (based on nutrient-addition experiments) and we expected SLA, LNC and LPC (indicators for a fast-growth strategy and high soil fertility) as well as Ellenberg N values (related to productivity), to explain a significant part of the variation in $N_L$. In contrast to our hypothesis, we found $N_L$ values of graminoids not to be different from these of other growth forms. A possible reason is that the amount of added hemiparasitic litter N was relatively low compared to N application rates in nutrient addition experiments. Also, most nutrient addition experiments use inorganic N that is readily available for plant growth, in contrast to litter (organic N). After exclusion of species with an alternative N strategy (legumes, ECM and ERM species), $N_L$ was positively related to SLA (both vegetation types) and to LNC and LPC (only at *Pedicularis* sites). On a global scale, high SLA and leaf nutrient concentrations are traits typical for
species with a fast-growth strategy associated with a quick return on investments (e.g.,
rapid leaf development, short leaf lifespan, little structural investments) adapted to a
high frequency of disturbances (Wright et al. 2004; Craine 2009). Moreover, in another
global analysis, SLA was related to proxies for N supply such as soil C:N and N
mineralization, and LNC and LPC are related to soil total N and/or P (Ordonez et al.
2009). Therefore, fast growing species (i.e., high nutrient demand) that are adapted to a
high soil fertility took up more N released from Rhinanthus and Pedicularis litter.
However, the association of species with site productivity (Ellenberg N-values) had no
significant effect on N\textsubscript{L} values. While this seems to contradict with the results of leaf
traits, an explanation could be that Ellenberg N values are only poorly related to the
availability of nutrients and more strongly to biomass production (Schaffers and Sykora
2000; Diekmann 2003). When (aboveground) disturbances are frequent, as is the case in
these mown grasslands, shoot biomass can be low at relatively high soil fertility, which
could explain the discrepancy between expectations and results. Our results add to the
rising awareness that SLA is one of the most meaningful determinants of plant strategies
(Westoby 1998; Diaz et al. 2004; Wright et al. 2004). We propose that parasitic infection
can be seen as a disturbance (e.g., burning, mowing, grazing), resulting in a decrease in
aboveground host (and often total) biomass and an increase in soil fertility of which non-
hosts and species with a fast-growth strategy profit (see also Quested 2008). The
decreased invasion resistance of grasslands parasitized by Rhinanthus alectorolophus
(Joshi et al. 2000) corroborates with the idea that hemiparasitic infection can be viewed
as disturbance.
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Average $N_L$ values of legume species were all below those modelled as a linear function of SLA (Figure 5.4). We suggest this is due to acquisition of (non-labelled) N from the air. On the other hand, the average $N_L$ values of ERM and ECM species were all above those modelled as a linear function of SLA, as well as LNC and LPC at *Pedicularis* sites. We speculate that these species possibly had better access to the N released from hemiparasitic litter by taking up organic N forms (e.g., amino acids) before mineralization to ammonium took place. Two to four months after litter addition, interspecific differences in $N_L$ values were more pronounced than 12 months after litter addition, mainly because of the drop in $N_L$ values of a few high-ranked species ($N_L > 3$). A possible explanation could be that these high-ranked species have little or no belowground nutrient storage, so that after mowing prior to litter addition nearly all nutrients were newly taken up from the soil. As mowing had been done in late autumn, in spring these plants had lost most of the initial $^{15}$N uptake (though they still ranked highest). Among low-ranked species, *Molinia caerulea* and *Juncus acutiflorus* are known to retranslocate nutrients very efficiently to their roots and rhizomes (Olff et al. 1994; Aerts 1996). After mowing, these species can rely on nutrient stores, and take up additional nutrients from the soil. This would explain smaller differences in $N_L$ between seasons.

5.4.4 Further research

*Rhinanthus* and *Pedicularis* often dominate the vegetation locally, but due to their annual and biennial life cycles, populations show high spatio-temporal variation (Petru 2005; Ameloot et al. 2006b). In mown semi-natural grasslands, their litter inputs can be a non-negligible source of nutrients (see chapter 2). Our results show that plant species
vary greatly in their ability to acquire this pulse supply of nutrients (25-30 fold differences in N$_L$). However, longer-lived hemiparasites not necessarily supply litter in pulses; for example, litterfall of the mistletoe *Amyema miquelii* and the hemiparasitic shrub *Exocarpus strictus* occurs throughout the year (March and Watson 2010; Watson et al. 2011). Differences in the seasonality of litterfall might result in markedly different N$_L$ values and, according to Craine (2005), may promote species with other sets of traits: to preempt a constant low supply of nutrients, species will have to maximize their root length rather than high growth rates or uptake capacity. Another important question is whether N – here used as an easy to trace proxy for nutrients in general – reflects the dynamics of other nutrients. March and Watson (2010) found that mistletoe infection (*Amyema*) increased annual litter nutrient returns to the soil in a temperate eucalypt forest by a factor of 1.65 (N), 3 (P) and 8.5 (K). We found a similar pattern in chapter 2: *Pedicularis* litter inputs contained 30%, 56% and 72% of aboveground vegetation N, P and K respectively. In contrast, *Rhinanthus* litter inputs contained similar proportions (9%, 9% and 10%) of aboveground vegetation N, P and K. If P and/or K are limiting plant growth at *Pedicularis* sites, the N$_L$ values reported in this chapter might greatly underestimate the true litter effect. In the light of the high atmospheric N deposition within the study region – about 2 g N m$^{-2}$ y$^{-1}$ (VMM 2009), similar to the amount of hemiparasitic litter N in this study (1.2-2.2 g N m$^{-2}$), (co)limitation by other nutrients is indeed likely. The amount of N derived from the hemiparasitic litter (N$_L$) was at highest 5.2% (*Rhinanthus*) and 6.2% (*Pedicularis*). Our study suggests that hemiparasitic litter has at least the potential to influence species composition in addition to the parasitic suppression of hosts species. To prove whether or not litter effects are able to alter
species composition, future research should perform long-term litter addition/removal experiments, and thereby focus on different nutrients.
Temperate semi-natural grasslands are biodiversity hotspots of global importance (Wilson et al. 2012) and their conservation and restoration are top priorities for conservation policy in Belgium and, by extension, the whole of Europe. The key role of parasitic plants – in particular root hemiparasitic *Orobanchaceae* – in structuring plant communities is widely recognized since several decades and was generally attributed to changes in competitive relationships between host and non-host species (parasitism pathway, Figure 1.1 in chapter 1). More recently, also litter effects have come into play (litter pathway, Figure 1.1 in chapter 1), by which high-quality hemiparasitic litter can increase plant-available nutrients in the soil and stimulate growth of both host and non-
host species. The relative importance of the litter pathway is expected to increase with decreasing nutrient status of the ecosystem (Spasojevic and Suding 2011). In this thesis, we performed a variety of studies to assess the litter and net community effects of two native hemiparasitic plant species growing in vegetation types with a contrasting nutrient status: *Rhinanthus angustifolius* C.C. Gmel. favoring mesotrophic grasslands and *Pedicularis sylvatica* L. growing in oligotrophic heath-grasslands. Here the results of chapters 2 to 5 are synthesized revisiting the original conceptual scheme of Figure 1.1 in chapter 1 (Figure 6.1). The litter pathway (point 6.1) and the net effect of weeding (point 6.2) are briefly discussed and compared between *Rhinanthus* and *Pedicularis*. Thereafter, we link chapters 3 and 5 by examining whether the litter effect favors host species (point 6.3). Towards the end of this chapter, we attempt to formulate some management implications (point 6.3) and suggestions for further research (point 6.4).

**Figure 6.1** Synthesis of the studied aspects of the litter pathway and the net effect of hemiparasites on the vegetation. **Litter pathway:** hemiparasitic litter amounts and decomposition dynamics (blue area, chapter 2), uptake of N from added hemiparasitic litter by co-occurring species (yellow area, chapter 5), and effect of litter on gross N transformation rates in the soil (dark area within yellow, chapter 4). **Net effect:** effect of continued hemiparasite weeding on biomass of growth forms and individual species (red area, chapter 2 and 3), and on species establishment (purple area, chapter 3)
6.1 Litter pathway

We expected litter effects to be more important in the oligotrophic Pedicularis sites compared to the mesotrophic Rhinanthus sites (see chapter 1). We discuss successively the amounts and decomposition dynamics of both hemiparasitic litter types (6.1.1), the effect of litter on soil N transformation rates (6.1.2) and litter N uptake by the vegetation (6.1.3).

6.1.1 Litter amount and decomposition

The litter pathway is driven by the hemiparasitic litter inputs to the soil. These consist of the dead part of the hemiparasite that is not removed by mowing. Rhinanthus litter inputs are only leaves – dead stems are removed by mowing (when leaves are shed); Pedicularis litter inputs are whole plants except the tops (ca. 20%) that are removed by mowing. Pedicularis litter returned twice as much N per square meter to the soil compared to Rhinanthus litter (see synthesis of results in Table 6.1). Also the amount of N released from Pedicularis litter remained considerably higher than for Rhinanthus over an 8-month period. The amount of Pedicularis litter N and its release expressed as a percentage of aboveground vegetation N content is two to three times higher compared to Rhinanthus litter. Moreover, Pedicularis litter contained surprisingly high phosphorus (P) and potassium (K) amounts relative to total aboveground vegetation (Table 2.4 in chapter 2). From these results we conclude that Pedicularis litter affects community composition and diversity potentially much more than Rhinanthus litter does. This is in line with our hypothesis that litter effects become more important when soil fertility is lower.
Chapter 6

**Table 6.1** Synthesis of the main results of this thesis. **Litter pathway**: litter amount and decomposition (chapter 2), soil N transformation rates (chapter 4), N uptake by the vegetation of N derived from hemiparasitic litter (chapter 5). **Net effect**: growth form biomass (chapter 2), species abundance and establishment chances (chapter 3). ***P < 0.001; * 0.01 < P < 0.05; NS not significant**

<table>
<thead>
<tr>
<th>Litter pathway</th>
<th>Rhinanthis (mesotrophic)</th>
<th>Pedicularis (oligotrophic)</th>
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<tr>
<td></td>
<td>(g N m⁻²)</td>
<td>(% of shoot N)</td>
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<td>8</td>
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<td><strong>Soil N transformation rates</strong></td>
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<td>(times litter mix)</td>
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<td>Flux from Norg to NH₄⁺</td>
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<td>x 1.6</td>
</tr>
<tr>
<td>Flux from NH₄⁺ to NO₃⁻</td>
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<td>x 1.8</td>
</tr>
<tr>
<td><strong>N uptake by vegetation</strong></td>
<td>(% of added litter N)</td>
<td>(% of shoot N)</td>
</tr>
<tr>
<td>Uptake 0-2 months</td>
<td>2.2</td>
<td>0.97</td>
</tr>
<tr>
<td>Uptake 4-12 months</td>
<td>1.8</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Net effect (weeding)</strong></td>
<td>(% shoot biomass increase)</td>
<td>(% shoot biomass increase)</td>
</tr>
<tr>
<td>Total</td>
<td>+24%</td>
<td>***</td>
</tr>
<tr>
<td>Total minus hemiparasite</td>
<td>+41%</td>
<td>***</td>
</tr>
<tr>
<td>Graminoid</td>
<td>+47%</td>
<td>***</td>
</tr>
<tr>
<td>Forb</td>
<td>+20%</td>
<td>*</td>
</tr>
<tr>
<td>Ericaceous shrub</td>
<td>-</td>
<td>+20%</td>
</tr>
<tr>
<td>Species</td>
<td>abundance</td>
<td>+/-</td>
</tr>
<tr>
<td>establishment</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### 6.1.2 Soil N transformation rates

The higher N release from *Pedicularis* litter compared to *Rhinanthus* litter (6.1.1) is only relevant if it also effectively increases plant-available N in the soil. Therefore, we quantified gross N transformation rates in control soil, soil amended with hemiparasitic litter and soil amended with a non-parasitic litter mix. This was done using ¹⁵N tracing model based on a laboratory incubation experiment (chapter 4). We found that the net flux from organic N to NH₄⁺ – a critical step in the N cycle – increased relatively more by *Rhinanthus* litter addition than by *Pedicularis* litter addition relative to both the control soil and the soil amended with the non-parasitic litter mix (Table 6.1). This seems to
contradict the results on community-level litter N amount and N release (6.1.1) – these were both higher for *Pedicularis* compared to *Rhinanthus*. It is difficult, however, to compare the results from the *in situ* study (6.1.1) with those of the laboratory incubation discussed here: the 1:600 litter:soil ratio (g/g) used in the incubation cannot be translated into a litter:area ratio as determined *in situ*. Yet, based on the faster initial %N release from *Pedicularis* litter (Figure 2.4b and in chapter 2) – which is independent of area – we expected stronger effects on N transformation rates measured within the first three weeks of decomposition. From these results we concluded that hemiparasitic litter has a significant effect on N transformation rates in the soil, and therefore on N cycling in general. Yet, how this relates to the *in situ* situation cannot be concluded. Therefore, we also measured the uptake of N derived from hemiparasitic litter by the vegetation (6.1.3).

### 6.1.3 N uptake by the vegetation

The increased soil N transformation rates following addition of hemiparasitic litter supports the idea that hemiparasitic litter inputs indeed increase plant-available N in the soil. To what extent hemiparasitic litter increases N availability to plants was studied by *in situ* addition of $^{15}$N labeled *Rhinanthus* and *Pedicularis* litter and subsequent tracing of $^{15}$N uptake in aboveground vegetation (chapter 5). We found that, after two months, total aboveground vegetation had obtained about 1% and 0.5% of its N from *Rhinanthus* or *Pedicularis* litter, respectively. For new growth the next season, this litter-derived N ($N_L$) was 0.5% for both vegetation types (Table 6.1). These results indicate that the contribution of hemiparasitic litter to the total vegetation N uptake was very limited. However, values for individual species differed more than an order of magnitude (up to
6.2%, see Figure 5.3 in chapter 5), and we found a correlation with leaf traits related to a fast growth strategy (Figure 5.4 in chapter 5). The low N_L values we found contradict at first sight with the established literature body that *Rhinanthus*-induced community change is facilitated by litter nutrient cycling. Fisher et al. (2013), for example, found that *Rhinanthus minor* litter increased total host community biomass by 10%. It is possible that the percentages of other nutrients plants derive from hemiparasitic litter (e.g., P_L or K_L values) are substantially higher than N_L values. In the case these other nutrients are (co)limiting, we can expect biomass to increase more than predicted by N_L-values. That being said, if the biomass increase induced by litter addition is proportional to a species’ N_L value, our results indicate the direction – not the size – of the community change to be expected. In and of itself, this fills an important knowledge gap. The higher N_L values for total aboveground vegetation at *Rhinanthus* sites compared to *Pedicularis* sites suggest that litter effects of *Rhinanthus* are more important than litter effects of *Pedicularis*. This finding is in agreement with the stronger effect of *Rhinanthus* litter on soil N transformation rates (Figure 4.3 in chapter 4), but contradicts its initially slower %N release and overall slower N release per square meter compared to *Pedicularis* litter from the litterbag experiment (Figure 2.4b-c in chapter 2). The discrepancy between the higher N release – per square meter – from *Pedicularis* litter and the smaller concurrent plant uptake compared to *Rhinanthus* litter can be explained by strong biotic immobilization and abiotic fixation in *Pedicularis* soil, as is suggested by the ^15^N tracing model (Figure 4.3 in chapter 4). This strong N fixation/immobilization in *Pedicularis* soil, presumably by soil organic matter (SOM), is most likely related to its low pH and relatively high C:N ratio (Table 4.1 in chapter 4) and a different microbial composition (e.g., higher fungi:bacteria ratio) (Eskelinen et al. 2009). Fungal-dominated
microbial food webs promote slow and highly conservative cycling of nutrients (Wardle et al. 2004).

6.2 **Net effects on the vegetation**

The net effect of hemiparasites on the vegetation depends on the relative importance of the parasitism and litter pathways. The effect of hemiparasitic litter was, in contrast to expectations, higher at mesotrophic *Rhinanthus* sites compared to oligotrophic *Pedicularis* sites. The net effect of *Rhinanthus* spp. on aboveground biomass is well-studied (Ameloot et al. 2005): *Rhinanthus* decreases total and graminoid biomass, while effects on forb biomass are variable. Yet, effects on individual species may not reflect that of the growth form they belong to (Mudrak and Leps 2010). For *Pedicularis*, no studies reported the net effect on the vegetation. We here discuss the net effect of *Rhinanthus* and *Pedicularis* weeding on aboveground biomass (6.2.1), species abundances (6.2.2) and species establishment (6.2.3).

6.2.1 **Aboveground biomass**

To study the net effect of hemiparasites on the vegetation, we set up an experiment in which *Rhinanthus* and *Pedicularis* were continuously weeded during three years and monitored biomass and species abundances. In agreement with literature (e.g., Ameloot et al. 2005), *Rhinanthus* weeding significantly increased total, total minus hemiparasite, graminoid and forb biomass (Table 6.1). On the other hand, *Pedicularis* weeding only increased total minus hemiparasite biomass significantly. This cannot be attributed to an effect of parasitism because it is a normal consequence of functional group removal that biomass of the remaining vegetation is increased (McLaren and Turkington 2011). Since *Pedicularis* litter N fulfilled only 0.5% of total aboveground vegetation N needs (6.1.3),
we suggest that the parasitism effects themselves are relatively low rather than being compensated by strong litter effects. Likewise, we suggest that the slightly higher litter effect of *Rhinanthus* was negligible in relation to its strong parasitism effects. Our results corroborate with those of Bardgett et al. (2006), who reported that *Rhinanthus minor* suppressed host community biomass despite a parasite-driven increase in N mineralization of 105-174%. Because of the low %N derived from *Rhinanthus* litter we found in the vegetation (6.1.3), we suggest that the strong increase in N mineralization they found – like the effect on N transformation rates we found (6.1.2) – did not substantially enhance the N uptake by the vegetation. However, hemiparasitic litter inputs accumulating over time might have stronger effects on the vegetation than our one-time addition of hemiparasitic litter suggests.

### 6.2.2 Species abundances

Weeding of *Rhinanthus* resulted in both ‘winner’ and ‘loser’ species (Table 6.1, Figure 3.3 in chapter 3), while weeding of *Pedicularis* yielded only winners. This is remarkable since *Pedicularis* weeding did not significantly increase aboveground biomass, whereas *Rhinanthus* did. This means that *Rhinanthus* mediates competitive relations to a larger extent than *Pedicularis* does: a strong increase in a limited number of heavily parasitized species not only compensates the decrease in a number of other (non-host) species, it increases the aboveground biomass of the vegetation.

The effect of hemiparasite weeding on individual species abundances did often not reflect the effect on the growth form they belong to. This is especially of interest for graminoids which are thought to be particularly vulnerable to parasitism as a group (e.g., Ameloot et al. 2005). *Agrostis* spp. and *Juncus acutiflorus* proved to be more vulnerable
to parasitism than *Anthoxanthum odoratum*, *Holcus lanatus*, *Luzula multiflora* and *Molinia caerulea*. We came up with a new hypothesis for species vulnerability to parasitism: the clonality hypothesis (see chapter 3). Species that possess clonal growth strategies – such as stolon formers or creeping species rooting at nodes – form extensive interconnected networks. When a ramet (an apparent individual) is parasitized, the parasite gains access to the whole clonal structure. Therefore, chances for encounters between parasite and host roots are higher. Species that significantly increased their biomass following hemiparasite removal generally possessed clonal growth, while those that decreased did not (Figure 3.3 in chapter 3).

6.2.3 Species establishment

Hemiparasites such as *Rhinanthus* spp. are thought to increase the chances for species establishment by decreasing sward density and ‘gap’ creation after the dying back. To test this hypothesis, we studied the effect of hemiparasite weeding on seed germination (chapter 3). Therefore, at the end of the second year of weeding, seeds of selected species were added to both weeded and non-weeded plots. While overall seed germination in the field was very limited, the germination of two species was negatively affected by weeding of both *Rhinanthus* and *Pedicularis*, while none of the species showed increased seed germination as a result of hemiparasite removal (Figure 3.4 in chapter 3). These limited results support our hypothesis that hemiparasites indeed increase establishment chances.
6.3 Does litter addition favor host species?

From our results, this important question is not easy to answer. However, for *Rhinanthus* sites we found a significant negative linear relation ($r = -0.66, P = 0.01$) between the N a species derived from the added hemiparasitic litter ($N_L$) and the effect of weeding on the same species (Figure 6.2).

![Figure 6.2](image)

*Figure 6.2* Nitrogen derived from hemiparasitic litter ($N_L$) in autumn (Figure 5.3 in chapter 5, full circles) as a function of the weeding effect (Figure 3.3 in chapter 3). For *Poaceae, Cyperaceae* and *Juncaceae*, $N_L$ values were calculated as the mean of individual species. The grey area represents the 95% confidence interval. A weeding effect of -1, 0 and 1 is equal to a decrease by 63%, no effect and an increase by 172%, respectively.

For *Pedicularis* sites we found no relation between these variables. The negative relation we found for *Rhinanthus* sites means that non-infected species, which are relatively promoted by the presence of the hemiparasite (and thus decreased by weeding of the hemiparasite) also profited most from the hemiparasite’s litter. Due to the small % N derived from hemiparasitic litter ($N_L$) in relation to the net effect of weeding (a value of
-1 is equivalent to a 63% biomass decrease), it is unlikely that the litter effect is responsible for the net effect of parasitism in the short-term. However, N_i values might as well underestimate the litter effect: litter addition – a source of labile C – might increase the decomposition of soil organic matter in soils with low N mineralization (Craine et al. 2007) and therefore increase N availability more than the N released from the litter alone.

6.4 Management implications

This thesis is in the first place a fundamental study rather than an applied one. Yet, here we attempt to translate our findings into practical management considerations.

Introduction of Rhinanthus spp. has been suggested as management tool to restore species-rich grassland after the cessation of fertilization (Pywell et al. 2004) as well as a means to reduce the biomass in road verges and thus their mowing frequency (Ameloot et al. 2006a). Our results support this idea as the species had strong negative effects on productivity, mainly of the dominant graminoid component. Moreover, Rhinanthus increased the number of germinated seeds of two sown species, increasing the potential for establishment of new species. But note that the sensitivity of Rhinanthus to drought stress and the resulting inter-annual variation in abundance of this annual hemiparasite (see Figure 3.2 in chapter 3) can hamper the applicability of Rhinanthus as a management tool. The effect of Rhinanthus on species abundances was highly variable and species with a clonal growth strategy suffered more, while species without clonal growth benefited more from parasitism. Short-term litter effects were small (6.1.3) and even in line with the effect of parasitism at the species level (Figure 6.2). However, more data is needed to confirm this finding. We propose that parasitic infection can be seen
as a disturbance (such as burning, mowing, grazing), resulting in a decrease in aboveground host (and often total) biomass and an increase in soil fertility of which non-hosts and species with a fast-growth strategy profit.

*Pedicularis*, on the other hand, seems less suited for the restoration of species-rich grassland in the sense that it had no significant effect on vegetation shoot biomass. Moreover, *P. sylvatica* is a species typically lost from oligotrophic meadows when nutrient loading increases or mowing ceases followed by an increase in biomass of stronger competitors (Leps 2005); therefore its introduction in previously fertilized habitats is questionable. Of course, there are other traits that matter in ecosystem restoration such as the attraction of bumblebees by *Pedicularis* (Bekker and Kwak 2005) as well as the temporal variation in species abundances induced by short-lived hemiparasites in ecosystems dominated by perennial plants (Figure 3.2 in chapter 3; Petru 2005; Ameloot et al. 2006b).

### 6.5 Perspectives for further research

While this thesis substantially improves our understanding of litter and net effects of hemiparasites on vegetation, our results also indicated several unresolved issues. The main issue is undoubtedly the lack of direct proof of the (long-term) litter effect. In several studies we focused on specific links of the litter pathway (part 6.1), yet we did not directly test its contribution to the net effect. In order to do so, we should have included parasitized plots with litter removal and unparasitized plots with litter addition. This approach was used – for the first time – for *Rhinanthus minor* using mesocosms of model grassland communities (Fisher et al. 2013). They included parasitism, litter and nutrient treatments in a fully factorial design. Litter addition and parasitism with litter
removal had opposite effects on total, grass and legume biomass: parasitism alone decreased biomass, whereas litter addition increased biomass. *R. minor* parasitism caused a greater reduction in total biomass in unfertilized communities than in fertilized communities. The next step is to perform similar studies in real plant communities. Because of the considerable inter-annual variation in the abundance of the hemiparasite (Figure 3.2 in chapter 3), long-term experiments are advisable. Also, treatments adding litter from co-occurring species could be added to compare hemiparasitic versus non-parasitic litter effects. Alternatively, future experiments could combine addition of $^{15}$N labeled hemiparasitic litter and $^{15}$N labeled non-parasitic litter mixtures to field plots and trace the subsequent $^{15}$N uptake by the vegetation.

Beside hemiparasitic litter inputs, also changes in the host community due to parasitism can change microbial community and therefore nutrient cycling. For example, parasitism can increase nutrient contents in the host community, improving the quality of host litter (e.g., Ameloot et al. 2008, Fisher et al. 2013) and reduce mycorrhizal associations (Press and Phoenix 2005). To tease out the effects of hemiparasitic litter and host community changes, nutrient cycling could be studied in a set-up similar to that of Fisher et al. (2013).

The inclusion of two nutrient levels in the full factorial parasitism x litter x nutrient experiment by Fisher et al. (2013) is a straightforward way of testing hypotheses about the influence of nutrient levels on the outcome of parasitism and litter effects. However, the effect of parasitism might not be independent from nutrient addition. Nutrient addition – increasing aboveground biomass – is likely to decrease the performance of the hemiparasite: *Rhinanthus angustifolius*, for example, could not establish in
grasslands with total aboveground biomass above 6 t ha\(^{-1}\) (Ameloot et al. 2006b). To avoid this, future research could focus on more hemiparasitic plant species along a natural gradient of productivity.

Future research should also focus on nutrients other than N, such as P and K. While *Rhinanthus* litter N:P:K ratio was balanced with that of the whole vegetation, *Pedicularis* contained relatively more P and especially K. A similar trend was reported for mistletoe litter inputs in a temperate eucalypt forest (March and Watson 2010). Therefore, our results for *Pedicularis* – based on N cycling alone – might underestimate the true potential of its litter effects on community composition and diversity. In addition, the rather high atmospheric N deposition within the study region (about 2 g N m\(^{-2}\)) (VMM 2009) suggests other nutrients might be limiting plant growth and therefore drive possible changes in species composition. Looking at vegetation and soil N:P ratios (Table 2.2 in chapter 2), P limitation is likely more important in *Pedicularis* sites than in *Rhinanthus* sites (Gusewell 2004). Phosphorus limitation at *Pedicularis* sites could explain, in part, why only such a small fraction of *Pedicularis* litter N is taken up by the vegetation (Table 6.1).

As the effect of hemiparasites on individual species is highly variable (6.2.2), even for species of the same growth form, more species-level studies should be performed. To test our clonality hypothesis in particular, future research could compare the impact of hemiparasites between intact clonal structures and clonal structures of the same species in which the conduct between individual ramets are interrupted.


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**R code for model selection**

First, we optimized the random structure of our model by comparing models with full fixed effects and different random structure using a Restricted Maximum Likelihood (REML) approach (Zuur et al. 2009). The model with the lowest Akaike Information Criterion (AIC) was retained. The weeding model was compared with an intercept-only model and the weeding*year model was compared with the weeding + year (without interaction effect) model using likelihood ratio tests. The fit of the final model was then checked by assessing normality and homogeneity using graphical tools.

**Effect of weeding of the hemiparasite on aboveground biomass in year 2010**

FULL MODEL: \( \text{biomass} \sim 1 + \text{weeding} + (1 \mid \text{location/block}) \)

**#STEP 1:** Selection of the random structure

\[
\begin{align*}
&M1 \leftarrow \text{lm}r(biomass \sim 1 + \text{weeding} + (1 \mid \text{location}), \quad \text{REML=T, data}) \\
&M2 \leftarrow \text{lm}r(biomass \sim 1 + \text{weeding} + (1 \mid \text{location/block}), \quad \text{REML=T, data}) \\
&\text{anova}(M1, M2)
\end{align*}
\]

**#STEP 2:** Testing for the weeding effect (random structure depending on previous step)

\[
\begin{align*}
&M10 \leftarrow \text{lm}r(biomass \sim 1 + \text{weeding} + (1 \mid \text{location}), \quad \text{REML=T, data}) \\
&M11 \leftarrow \text{lm}r(biomass \sim 1 + (1 \mid \text{location}), \quad \text{REML=T, data}) \\
&\text{anova}(M10, M11)
\end{align*}
\]

**Effect of year:weeding interaction on aboveground biomass for 2009 and 2010 data combined**

FULL MODEL: \( \text{biomass} \sim 1 + \text{weeding} + \text{year} + \text{weeding:year} + (\text{year-1} \mid \text{location/block}) + (1 \mid \text{plot}) \)

**#STEP 1:** Selection of the random structure

\[
\begin{align*}
&M1 \leftarrow \text{lm}r(biomass \sim \text{weeding} + \text{year} + \text{weeding:year}+(1 \mid \text{plot}), \quad \text{REML=T, data}) \\
&M2 \leftarrow \text{lm}r(biomass \sim \text{weeding} + \text{year} + \text{weeding:year}+(\text{year-1} \mid \text{location})+(1 \mid \text{plot}), \quad \text{REML=T, data}) \\
&M3 \leftarrow \text{lm}r(biomass \sim \text{weeding} + \text{year} + \text{weeding:year}+(\text{year-1} \mid \text{location/block})+(1\mid\text{plot}), \quad \text{REML=T, data}) \\
&\text{anova}(M1, M2, M3)
\end{align*}
\]

**#STEP 2:** Testing for the weeding effect (random structure depending on previous step)

\[
\begin{align*}
&M30 \leftarrow \text{lm}r(biomass \sim \text{weeding} + \text{year} + \text{weeding:year}+(\text{year-1} \mid \text{location})+(1 \mid \text{plot}), \quad \text{REML=F, data}) \\
&M31 \leftarrow \text{lm}r(biomass \sim \text{weeding} + \text{year} + (1 \mid \text{location})+(1 \mid \text{plot}), \quad \text{REML=F, data}) \\
&\text{anova}(M30, M31)
\end{align*}
\]
Figure B.1 Measured (mean ± SE) and modeled (dashed lines) NH$_4^+$ (a-c) and NO$_3^-$ (d-f) concentrations as a function of time after $^{15}$N label addition in the three Rhin-D treatments: control soil (a, d), soil amended with a non-parasitic litter mix (b, e) and soil amended with Rhinanthus litter (c, f).
Figure B.2 Measured (mean ± SE) and modeled NH$_4^+$ (a-c) and NO$_3^-$ (d-f) concentrations as a function of time after $^{15}$N label addition in the three Pedi-H treatments: control soil (a, d), soil amended with a non-parasitic litter mix (b, e) and soil amended with Pedicularis litter (c, f).
Curriculum vitae

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Demey A, Rütting T, Huygens D, Staelens J, Boeckx P, Hermy M, Verheyen K (under review) Litter pathway of hemiparasitic plants confirmed by gross soil N dynamics, Soil Biology and Biogeochemistry (IF = 3.504)


Scientific reports


Publications in proceedings of scientific congresses

Curriculum vitae

MSc thesis

Demey A (2008) Koppelen van de koolstof-en stikstofcyclus in subarctische toendra, MSc thesis, Ghent University, Ghent, Belgium

Scientific activities

Participation in symposia with oral presentation


Demey A, Ameloot E, Boeckx P, Hermy M, Verheyen K (2011) Hemiparasitic plants affect community structure and biogeochemical cycling. Oral presentation at 41st Annual Meeting of Ecological Society of Germany, Austria and Switzerland (GfÖ), 5-9 September 2011, Oldenburg, Germany


Participation in symposia with poster presentation


Appendix C

grasslands in Flanders. Poster presentation at Annual symposium of the British Ecological Society (BES), 20-22 April 2009, Aberdeen, Scotland, UK

Participation in symposia without presentation

23-24 November 2011 International conference forests 2011, Leuven, Belgium
15-16 April 2010 BASIS annual meeting, Arnhem, The Netherlands
18 March 2010 Starters in het natuuronderzoek, Brussels, Belgium
2-3 April 2009 BASIS annual meeting, Bruges, Belgium

Supervision of MSc thesis students

2011-2012 Robin Van Heghe: Impact van de halfparasieten *Rhinanthus angustifolius* en *Pedicularis sylvatica* op de vegetatie in halfnatuurlijke graslanden. Supervisors: Prof dr. Dries Bonte en Prof dr. ir. Kris Verheyen

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