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NITROGEN CYCLING AND SEQUESTRATION IN TEMPERATE FOREST EDGES

Thesis submitted in fulfillment of the requirements
for the degree of Doctor (PhD) in Applied Biological Sciences:
Forest and Nature Management

Dutch translation of the title:
De stikstofdynamiek en –vastlegging in randen van gematigd bos

Illustration on the front cover:
Forest edge in an agricultural landscape

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Woord vooraf

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Table of Content

Summary

The European landscape is characterized by small forest remnants due to a long history of land-use change, particularly in the lowlands. Forest edges differ from forest interiors in terms of e.g. microclimate, atmospheric deposition and biodiversity. Despite several directives and policies aiming to reduce the use and emission of nitrogen (N), anthropogenic activities still give rise to high atmospheric N deposition levels. Forest edges are subjected to higher atmospheric deposition levels compared to forest interiors, making them potentially more prone to eutrophication, acidification, N loss (via leaching or in gaseous forms) and species loss. However, forest edges challenge the current N-saturation paradigm, as Wuyts et al. (2011) measured a local decline in nitrate (NO_3^-) seepage within the first 20 m from the edge.

It is still unclear how elevated atmospheric N deposition specifically affects N and carbon (C) stocks and cycling at temperate forest edges. The specific aims of this thesis were (i) to assess the edge effect on N stocks, C stocks and their sequestration and (ii) to determine which processes of the forest N cycle differ between forest edge and interior. An edge-to-interior transect was laid out in six temperate oak (*Quercus robur* L.), pine (*Pinus nigra* ssp. *nigra* Arnold and *P. nigra* ssp. *laricio* Maire) and spruce (*Picea sitchensis* (Bong.) Carr.and *P. abies* (L.) Karst) forests in northern Belgium (Flanders) and Denmark growing on acid, sandy quartz-dominated Podzols from which data on N throughfall and leaching were available from previous research. All forest edges border arable land dominated by intensive livestock production and have experienced several decades of elevated N deposition.

Total N stocks, total C stocks and soil C sequestration were increased at the forest edge compared to the interior. More specifically, N and C stocks in wood, roots (coarse and fine) and mineral soil were increased at the forest edge. As N deposition is higher in coniferous forests compared to deciduous forests, a more pronounced edge effect in the pine and spruce stands than in the oak stands was expected. However, this was not the case in this study, signifying that the edge effect is not solely driven by forest type, but more likely the result of an interplay of several factors (landscape matrix, edge structure, height, age, leaf area index (LAI)). We hypothesized that the lower N and C stocks in the forest floor but higher N and C stocks in mineral soil at the edge

were due to a faster litter degradation (as a result of differences in microclimate and soil micro- and macrofauna at edge vs. interior) hereby transferring nutrients to deeper soil layers.

Litter decomposition and nutrient release were assessed via the litterbag technique. Increased litter mass loss and nutrient release were observed at the edge compared to the interior in the oak stands, which were governed by soil acidity and forest floor C/N ratio. In the pine stands, only release of N and exchangeable cations (EC, sum of calcium, Ca^{2+}, magnesium, Mg^{2+} and potassium, K^{+}) was higher at the edge compared to the interior. Several factors can drive litter decomposition, such as litter position, litter quality and presence of the soil decomposer community. The influence of litter position and litter quality was examined via the interchange of edge and interior litter, while the influence of the specific edge arthropod detritivores was assessed via placing interior litter in open top chambers (OTC), which create a warmer 'edge' microclimate in the interior. Edge conditions (microclimate, atmospheric deposition, soil fauna and soil physicochemical properties), litter quality and edge arthropod detritivores all influenced litter decomposition and nutrient release, but the contribution of each driving factor depended on the specific edge characteristics of each site.

The microbial community was mapped via the extraction of phospholipid fatty acids (PLFA) and aminosugars (AS) from the upper mineral soil layer (0 – 10 cm). Biomass of Gram positive (Gram+) bacteria was higher at the forest edges compared to the forest interiors. Nitrogen cycling rates (mineralization and nitrification) were assessed via the ^{15}N pool dilution technique in mineral soil. Gross mineralization rates were stimulated at the warmer forest edges and were associated to the higher bacterial biomass at the forest edges. Nitrification rates were not affected by edge proximity, but differed between forest types, as the oak stand was characterized by higher nitrification rates than the pine and spruce stands. A ^{15}N tracer study was used to explore N retention in the different soil layers (litter, fermentation and humus layer and mineral soil) over time. In all forest types, the forest interior retained more N in the litter layer, while N was stored in deeper soil layers at the edge.

Automatic measuring chambers were used to monitor fluxes of N and C trace gases during a two-week measurement campaign in the oak and pine stand. Forest edges

emitted on average 60 % less nitric oxide (NO) and took up 177 % more methane (CH_4) at the oak site. Fluxes of nitrous oxide (N_2O) did not differ between forest edge and interior. Contrary to the postulated hypotheses, increased N deposition at the edges did not stimulate emission of NO or N_2O and did not inhibit uptake of CH_4. Instead, the contrasting microclimate at the forest edge influenced N and C trace gas fluxes as soil moisture variation between forest edge and interior was a key variable explaining the magnitude of NO, N_2O and CH_4 fluxes.

In conclusion, forest edges significantly influenced N and C stocks, cycling and sequestration. The studied forest edges stored large amounts of N and C (in above- and belowground biomass and soil) and showed increased N cycling rates, while the oak forest edges also emitted less NO and took up more CH_4 than forest interiors. Forest edges can play a currently still overlooked role in climate change mitigation via surplus C and N sequestration compared to forest interiors. However, it remains unclear for how long forest edges can sequester additional N and C under ongoing high N deposition and to what extent the sequestration depends upon the environmental context (soil type, climate, etc.). Therefore, more research in temperate forest edges is needed to provide an adequate knowledge of their N and C storage capacity and long-term behavior, which is imperative to obtain correct present and future forest N and C budgets on a larger (regional, national or global) scale.

Samenvatting

Door verandering van landgebruik beslaan bosfragmenten en bosranden een groot deel van het huidige Europese landschap, vooral in lager gelegen gebieden. Bosranden verschillen van boskernen op vlak van o.a. microklimaat, atmosferische depositie en biodiversiteit. Ondanks verscheidene beleidsmaatregelen die het gebruik en de uitstoot van stikstof (N) willen beperken, kunnen antropogene activiteiten nog steeds aanleiding geven tot hoge atmosferische N-depositiewaarden. Bosranden worden gekenmerkt door een hogere atmosferische depositie dan boskernen. Deze verhoogde input van N aan de bosrand kan de interne N-cyclus verstoren en potentieel zorgen voor eutrofiëring, verzuring, N-verliezen (via uitspoeling of gasvormige verliezen) en verlies aan biodiversiteit. In bosranden wordt de verhoogde doorval echter niet gereflecteerd in de N-verliezen, want Wuyts et al. (2011) maten een lagere uitspoeling van nitraat (NO_3^-) binnen een afstand van 20 m van de bosrand ondanks de verhoogde N-depositie.

Het is nog onduidelijk hoe de verhoogde N-depositie aan de bosrand van gematigde bossen de N- en koolstof (C) cyclus beïnvloedt. De specifieke doelen van deze thesis waren (i) het onderzoeken van het bosrandeffect op de N-voorraden, C-voorraden en hun vastlegging en (ii) bepalen welke processen van de N-cyclus verschillen tussen bosrand en boskern. Een rand-kern transect werd hiervoor uitgelegd in zes gematigde eiken- (*Quercus robur* L.), dennen- (*Pinus nigra* ssp. *nigra* Arnold and *P. nigra* ssp. *laricio* Maire) en sparrenbestanden (*Picea sitchensis* (Bong.) Carr.and *P. abies* (L.) Karst) in Vlaanderen en Denemarken, De bossen kwamen allemaal voor op arme, zure zandgronden waarvan reeds data over N-doorval en -uitspoeling beschikbaar waren. De bosranden grensden aan landbouwgronden, beheerd door veeteeltbedrijven en worden reeds enkele decennia gekenmerkt door een hoge N-depositie.

De totale N- en C-voorraden waren hoger aan de bosrand ten opzichte van de boskern, net als de C-vastlegging in de bodem. Wanneer we naar de verschillende compartimenten van het bos kijken, waren de voorraden van het hout, de wortels (grove en fijne) en de minerale bodem significant verhoogd aan de bosrand. Omdat de depositie van N hoger is in naaldbossen ten opzichte van loofbossen, werd een meer uitgesproken randeffect verwacht in de onderzochte dennen- en sparrenbestanden in

vergelijking met de eikenbestanden. Dit was echter niet het geval, waardoor duidelijk werd dat het randeffect niet enkel van het bostype afhangt, maar eerder van een interactie van verscheidene factoren (het omliggende landschap, de structuur van de bosrand, de boomhoogte, de leeftijd, de bladoppervlakte). We stelden als hypothese dat de lagere N- en C-voorraden in de organische bodemlaag, maar hogere N- en C-voorraden in de minerale bodemlaag te wijten waren aan een snellere strooiselafbraak aan de bosrand (als gevolg van de verschillen in microklimaat en bodemfauna tussen bosrand en –kern), waarbij nutriënten sneller in diepere bodemlagen terecht komen.

De strooiselafbraak en het bijhorende verlies aan nutriënten werd opgevolgd via strooiselzakjes (*litterbags*) en verliep sneller in de bosrand ten opzichte van de kern in de eikenbestanden. Strooiselafbraak en het bijhorende verlies aan nutriënten bleek gerelateerd te zijn aan de zuurtegraad van de bodem en de C/N ratio van de organische bodemlaag. In de dennenbestanden was enkel de vrijstelling van N en uitwisselbare kationen (de som van calcium, Ca^{2+}, magnesium, Mg^{2+} en kalium, K^{+}) hoger aan de bosrand ten opzichte van de kern. Verschillende factoren, zoals de locatie, de kwaliteit van het strooisel en de aanwezigheid van bodemfauna beïnvloeden strooiselafbraak. De impact van locatie en strooiselkwaliteit werd onderzocht via de uitwisseling van rand- en kernstrooisel. De invloed van de specifieke arthropodengemeenschap aan de bosrand werd achterhaald via het plaatsen van kernstrooisel in *open top* kamers, die een warmer 'bosrand' microklimaat creeëren in de boskern. De condities aan de bosrand (microklimaat, atmosferische depositie, bodemfauna en fysicochemische bodemcondities), de strooiselkwaliteit en de arthropodengemeenschap aan de rand hadden elk een impact op de strooiselafbraak en het nutriëntenverlies, maar de bijdrage van elke factor hing af van de specifieke randkarakteristieken van elk bestand.

De microbiële gemeenschap werd in kaart gebracht via de extractie van vetzuren (uit fosfolipiden, PLFA) en aminosuikers (AS) uit de minerale bodem (0 – 10 cm). De biomassa van Gram positieve (Gram+) bacteriën was hoger aan de bosrand. De transformatie van N (via mineralisatie en nitrificatie) werd bestudeerd via de dilutie van het zwaardere ^{15}N-isotoop (*^{15}N pool dilution technique*) in de minerale bodem. Mineralisatie verliep sneller aan de warmere bosrand en was geassocieerd met de hogere bacteriële biomassa die er opgemeten was. Nitrificatie bleek niet beïnvloed te

zijn door het randeffect, maar wel door bostype, aangezien nitrificatie hoger was in de eikenbestanden in vergelijking met de dennen- en sparrenbestanden. Via het sproeien en opvolgen van ^{15}N in de tijd (*^{15}N tracer study*) kon de retentie van N in de verschillende bodemlagen (strooisel, humuslaag en minerale bodem) opgevolgd worden. Stikstof werd in de boskern vastgehouden in de strooisellaag, terwijl N vooral teruggevonden werd in diepere bodemlagen aan de bosrand.

Het bosrandeffect op de uitstoot van N- en C-gassen werd bestudeerd via automatische meetkamers tijdens een meetcampagne van twee weken in een eiken- en dennenbestand. In het eikenbestand stootte de bosrand minder stikstofmonoxide (NO) uit en nam meer methaan (CH_4) op dan de boskern. De uitstoot van distikstofoxide (N_2O) verschilde niet tussen bosrand en –kern. In tegenstelling tot de gestelde hypotheses heeft de verhoogde N-doorval aan de rand de uitstoot van N-gassen niet gestimuleerd en de opname van CH_4 niet geïnhibeerd. Het contrasterende microklimaat van bosrand en –kern bleek de fluxen van N- en C-gassen sterk te beïnvloeden, aangezien het bodemvochtgehalte een sterke drijver was van de gemeten NO-, N_2O- en CH_4-fluxen.

Uit dit onderzoek bleek dat bosranden een significante invloed hebben op de N- en C-cyclus in bossen. De bestudeerde bosranden sloegen meer N en C op (zowel in bovengrondse als ondergrondse biomassa en in de bodem) en vertoonden snellere N-transformaties (via decompositie en mineralisatie). Bovendien stootte de rand van het eikenbestand minder NO uit en nam meer CH_4 op dan de boskern. Bosranden kunnen een belangrijke rol spelen in het tegengaan van de klimaatverandering via de hogere C- en N-vastlegging in vergelijking met de boskern, wat tot nu toe nog onvoldoende onderzocht werd. Het blijft echter onzeker in hoeverre bosranden extra N en C kunnen opslaan onder aanhoudende hoge N-deposities en hoe sterk deze vastlegging afhankelijk is van de omgeving (bodemtype, klimaat, enz.). Meer onderzoek in gematigde bosranden is nodig om de correcte opslagcapaciteit voor N en C te berekenen en inzicht te verkijgen in hun lange termijn gedrag. Deze data zijn noodzakelijk om niet alleen de huidige, maar ook de toekomstige N- en C-budgetten correct in te schatten op grotere (regionale, nationale of globale) schaal.

List of abbreviations and symbols

Abbreviations

^{15}N	Stable isotope of nitrogen with mass 15
$^{15}N_{rec}$	Percentage of ^{15}N tracer recovered
AIC	Akaike information criterion
AS	Amino sugars
CL	Critical load
C_{resp}	Carbon sequestration response to nitrogen deposition (kg C kg^{-1} N)
C_{seq}	Carbon sequestration (kg ha^{-1} yr^{-1})
dbh	Diameter at breast height
DNRA	Dissimilatory reduction of nitrate to ammonium
EEA	European Environment Agency
FAA	Free amino acids
FH	Fermentation and humus layer
Gal	Galactosamine
GHGs	Greenhouse gases
Glu	Glucosamine
Gram+	Gram positive bacteria
Gram-	Gram negative bacteria
ICC	Intraclass correlation coefficient
L	Litter layer
LAI	Leaf area index (m^2 m^{-2})
LMA	Leaf dry mass per area (kg m^{-2})
MS10	Mineral soil of 0 to 10 cm deep
MS20	Mineral soil of 10 to 20 cm deep
Mur	Muramic acid
OTC	Open top chambers
Pa	Stand dominated by *Picea abies* (L.) Karst
PLFA	Phospholipid fatty acids
Pn	Stand dominated by *Pinus nigra* ssp. *laricio* Maire or *P. nigra* ssp. *nigra* var.*nigra* Arnold
Ps	Stand dominated by *Picea sitchensis* (Bong.) Carr.
Qr	Stand dominated by *Quercus robur* L.
R^2	Coefficient of determination
SOM	Soil organic matter

Chemical compounds

C	Carbon
Ca^{2+}	Calcium
CH_4	Methane
CO_2	Carbon dioxide
DIN	Dissolved inorganic nitrogen

DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EC	Exchangeable cations (= $K^+ + Mg^{2+} + Ca^{2+}$)
K^+	Potassium
Mg^{2+}	Magnesium
N	Nitrogen
N_2	Nitrogen gas
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Oxygen
O_3	Ozone
P	Phosphorus
SOC	Soil organic carbon

Symbols

k	Decomposition rate according to Olson's single exponential model
p	Significance of statistical test
r	Pearson correlation coefficient
r_s	Spearman rank correlation coefficient
t	test statistic (t-value)

1. Introduction

Central and Western Europe are characterized by small forest remnants resulting from a long-term history of land-use change (Decocq et al., 2016; Hofmeister et al., 2013). A co-occurring trend during the last decades was the considerable increase in atmospheric nitrogen (N) load as a result of anthropogenic activities (Duprè et al., 2010). This increased N deposition can have harmful effects on natural ecosystems, such as eutrophication (Gundersen et al., 1998a, 1998b), acidification (De Schrijver et al., 2012) and species loss (De Schrijver et al., 2011). Moreover, forest edges are subjected to higher atmospheric and throughfall deposition levels compared to forest interiors (e.g. De Schrijver et al., 2007; Wuyts et al., 2008a, 2008b) and therefore more subject to the effects listed above.

Since the 1990's, several directives and policies aimed to reduce the use and emission of gaseous N compounds (www.eea.europa.eu). However, in severely anthropized landscapes, N deposition levels can still be high. It is still unclear how this elevated atmospheric deposition specifically affects N and carbon (C) stocks and cycling at temperate forest edges. Therefore, the aim of this study is to scrutinize the effect of edge proximity on N and C biogeochemistry.

In this introductory chapter, facts and figures on landscape fragmentation and N deposition in Europe and Flanders (northern Belgium) are shown. Furthermore, the effects of elevated N input on forests are discussed, together with the different soil processes within the forest N cycle. Next, the characteristics of forest edges, i.e. how they differ from the forest interior, are specified. The link between the N and C cycle is addressed to clarify the role of forests as N and C sinks or sources. Finally, a schematic overview of the aims of this thesis is presented.

1.1. Forest fragmentation in Europe and Flanders

1.1.1. Forest fragmentation in Europe

The European Union (EU) currently contains 5 % of the world's forests, which equals an area of 155 million ha. As a result of afforestation programmes and natural succession of abandoned farmland, the area covered by forests in the EU-27 (27 member states) has increased at a rate of approximately 0.4 % per year since 1990 (http://europa.eu, Memo European Commission, 2013). However, within the EU-27, 40 % of the forests are within a distance of 100 m from other land use types and are therefore not regarded as forest interior

but as forest edges. When looking at the whole EU (38 countries), the percentage of forests within a distance of 100 m from other land use types ranges from 20 to 70 % depending on the country (Estreguil et al., 2013). Forest connectivity can be defined by the distance between forest patches, as it refers to the degree to which landscapes facilitate or impede the movement of species. Forest fragmentation leads to isolation of different forest patches, reducing the capability of organisms to move from one patch to the other and interfering with pollination, seed dispersal, wildlife migration and breeding. The European-wide map in Figure 1.1 provides the degree of forest connectivity per landscape unit of 25 by 25 km and for forest species with an average dispersion of 1 km. Forest patches as small as 1 ha were accounted for in the connectivity assessment. A value of 100 % indicates all forests are maximally connected and a percentage lower than 30 % represents isolated, poorly connected forests (light green colour code in Fig. 1.1). Landscapes with isolated, poorly connected forests represent up to 70 % of the European territory and are potentially more vulnerable to further fragmentation in the future. Consequently, forest edges and small forest remnants have become important features in the landscape (Decocq et al., 2016; Harper et al., 2005).

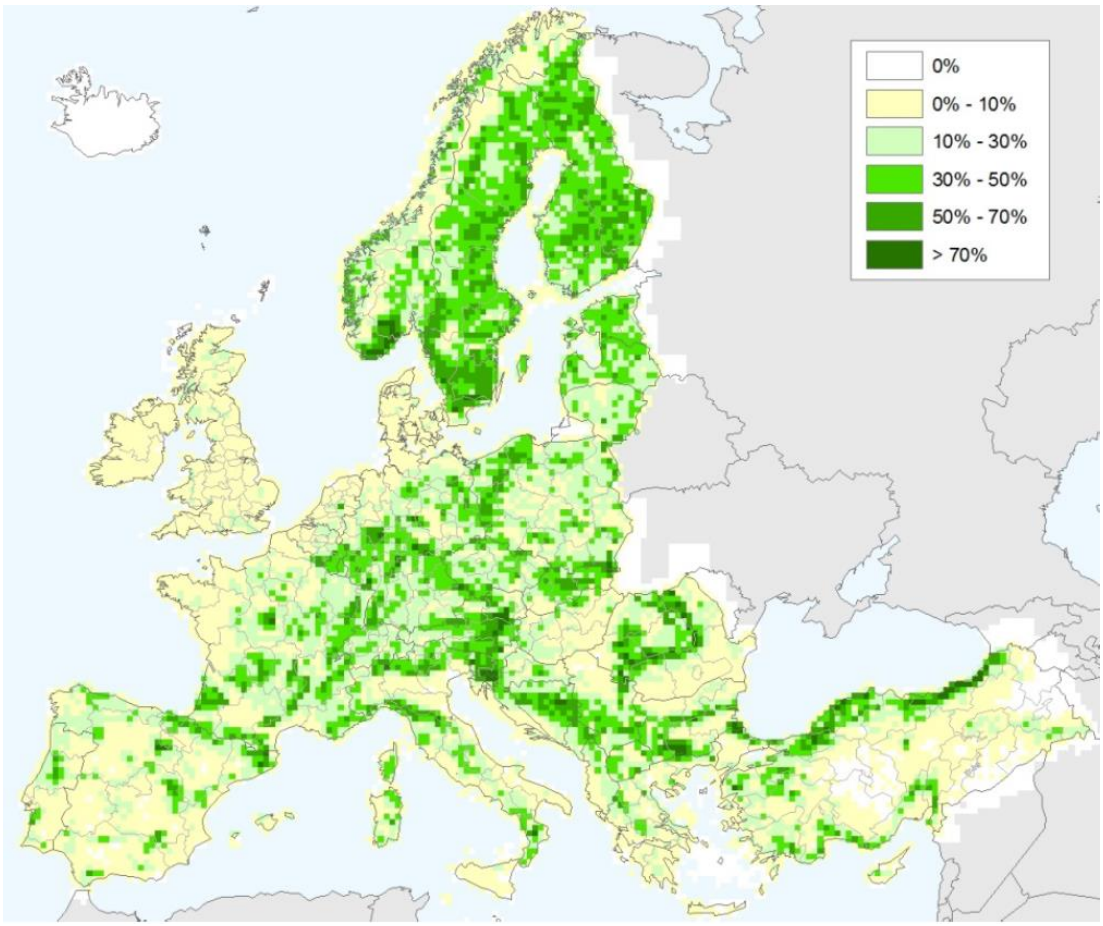

Fig. 1.1: Forest connectivity in Europe (38 countries) based on landscape units of 25 by 25 km and for forest species with an average dispersion of 1 km (Estreguil et al., 2013).

1.1.2. Forest fragmentation in Flanders (northern Belgium)

In Flanders, forest covers an area of 145 000 ha (Fig. 1.2) and most forests occur on poor, sandy soils in the Campine region, in the provinces of Antwerp and Limburg (Afdeling Bos & Groen, 2001).

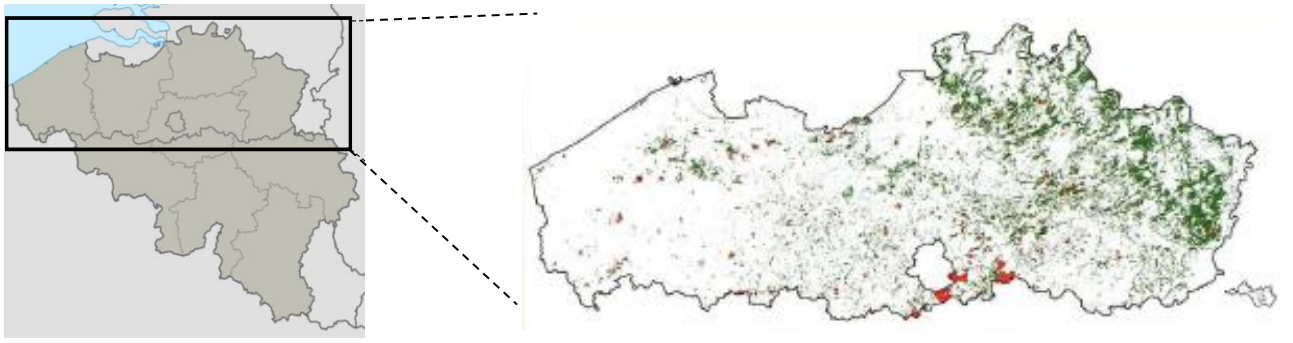

Fig. 1.2: Belgium (dark grey) with Flanders delineated by the black box. The red area indicates forested area since 1775, the green area indicates the current area occupied by forests, but was not permanently forested between 1775 and 2004 (adapted from Hermy and Vandekerkhove, 2004).

Flanders is a highly urbanized region with an average population density of about 355 inhabitants per square kilometer (NSI, 2010). The impact of urbanization, agriculture and transport infrastructure upon the landscape is severe (Van Eetvelde and Antrop, 2011), resulting in small, scattered forests diverse in composition and structure. About 85 % of the forests in Flanders is smaller than 5 ha (Hermy and Vandekerkhove, 2004). De Schrijver et al. (2007) calculated that 58 % of the forested area in Flanders consists of external forest edges (bordering non-forested land), when considering a distance of 50 m into the forest to represent the forest edge.

1.2. Nitrogen deposition in Europe and Flanders

In Europe, highest mineral N deposition values (> 35 kg ha^{-1} $year^{-1}$ of N) coincide with intensive livestock breeding areas (MIRA 2011), such as Flanders. The modelled N (NH_x + NO_x) deposition for Europe in 2009 can be seen in Fig. 1.3, where northern Belgium (Flanders), the Netherlands, northern Germany, Brittany (France) and northern Italy are subjected to the highest N deposition levels.

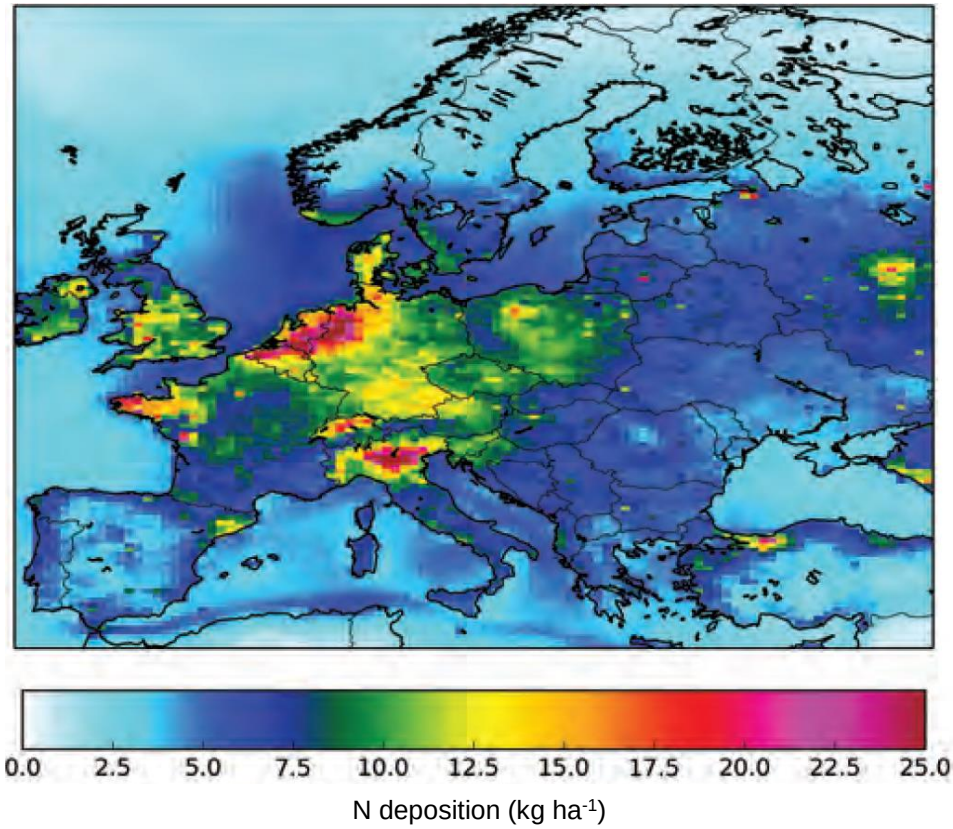

Fig. 1.3: Modelled N (NH_x + NO_x) deposition (kg N ha^{-1}) for Europe in 2009 (Erisman et al., 2015).

In Flanders, the total yearly mineral N deposition (wet and dry, see § 1.3) was as high as 49 kg ha^{-1} in 1990. It has decreased since then, but still averaged to no less than 32 kg ha^{-1} in 2011. Most forests of mid to high latitudes were N-limited until the 1950's, but due to a high atmospheric N load during the last decades this has changed considerably (Duprè et al., 2010). Erisman et al. (2015) presented an overview of the achievements and the current state of knowledge on reactive N in Europe, focusing on deposition, exceedances, and modelled and measured trends. Between 1990 and 2014, nitrogen oxides (NO_x) and ammonia (NH_3) emissions in Europe's EEA member countries (EU-28 and Iceland, Norway and Liechtenstein) declined by 51 and 11 %, respectively (Fig. 1.4). These decreases are mainly due to policies that enforced measures in transport and fuel switching, improvement in the energy and production industries, and the Nitrate Directive reducing the use of N fertilizers (EEA technical report 2014).

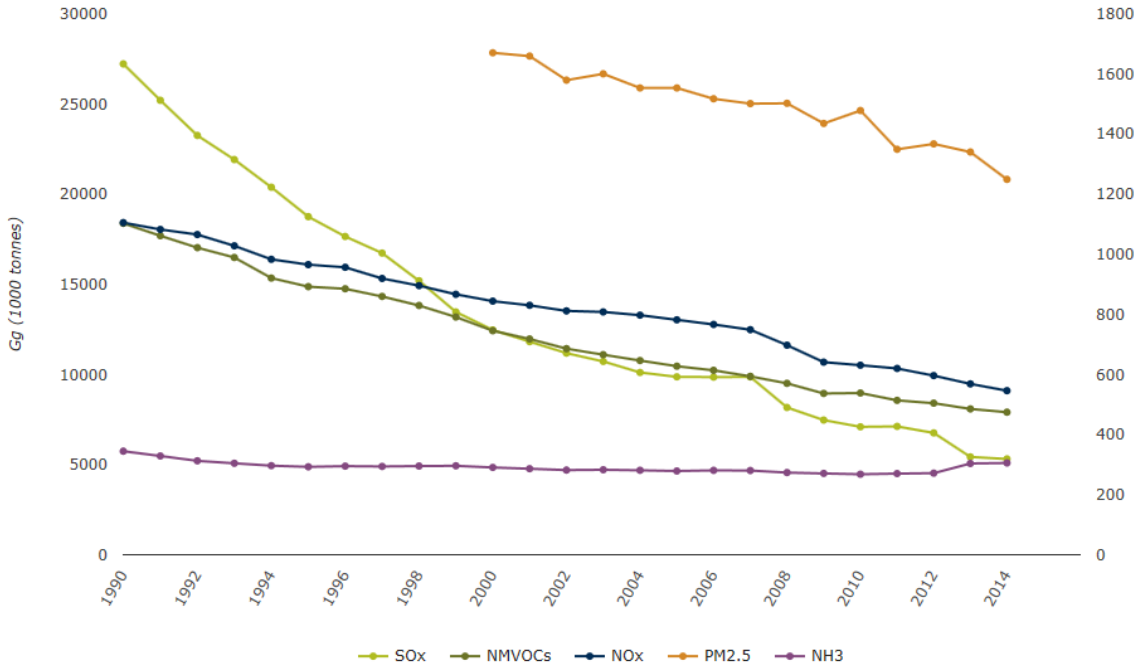

Fig. 1.4: Emissions trends of the air pollutants sulphur oxides (SO_x), nitrogen oxides (NO_x), ammonia (NH_3), non-methane volatile organic compounds (NMVOCs) and primary fine particulate matter ($PM_{2.5}$) from 1990 to 2014 (2000 to 2014 for PM) in the EEA member countries (EU-28 and Iceland, Norway, Liechtenstein). $PM_{2.5}$ emissions are shown on the secondary y-axis (EEA technical report 2014).

The 'quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge' (Nilsson and Grennfelt, 1988) is defined as the critical load (CL). Figure 1.5 shows the exceedance of the critical load due to N deposition in Europe for 2010, proving a high exceedance of the CL (more than 1 200 equivalents or 16.8 kg N ha^{-1} yr^{-1}) in northern Belgium. The exceedance of the CL is expressed in equivalents, which is a way of expressing the amount of an element by taking into account its atomic weight and valence. The results were computed using the 2015 Critical Loads database hosted by the Coordination Centre for Effects (CCE, www.eea.europa.eu). Empirical critical loads for N deposition (kg N ha^{-1} yr^{-1}) to natural and semi-natural ecosystems have been established by Bobbink et al. (2015), with a CL of 10 to 20 kg of N ha^{-1} yr^{-1} on mixed temperate forests. Overall, using the 2015 CL database, the European ecosystem area at risk of excessive N deposition is 75 % in the EU (Slootweg et al., 2015). Wuyts et al. (2009a) have shown that when only forest interior deposition was considered the average exceedance of the CL for N was 18 to 26 % lower than when edge deposition was accounted for in five regions of Flanders.

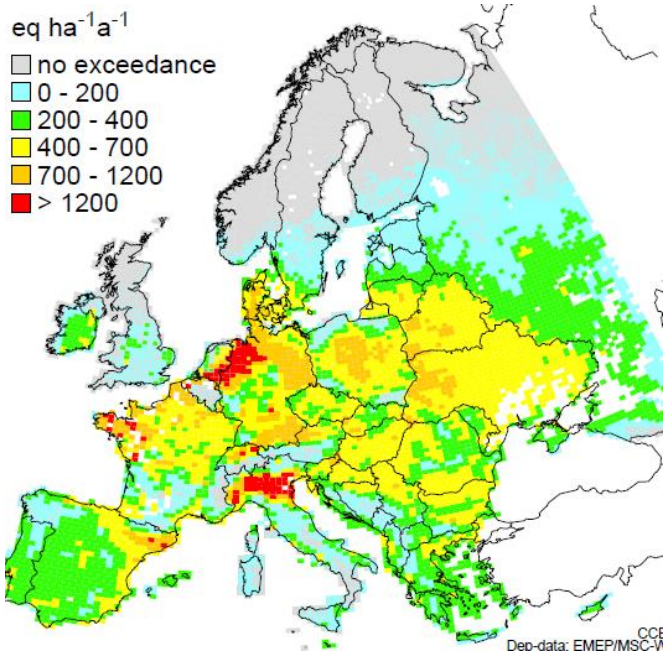

Fig 1.5: The average accumulated exceedance by total mineral nitrogen (N) deposition in 2010 using the European critical loads (CL) database of 2015 (www.eea.europa.eu).

In 1992, the Habitats Directive was adopted to conserve natural habitats and wild fauna and flora. Together with the Birds Directive, it forms the basis of Europe's nature conservation policy by establishing the Natura 2000 ecological network of protected areas (www.natura2000.vlaanderen.be). In 2014, the Flemish government agreed on 36 targets (Dutch: 'instandhoudingsdoelstellingen', IHD) necessary to protect sensitive habitats and species in designated Special Areas of Conservations and Special Protection Areas, as respectively instructed by the Habitats and Birds Directive. Each activity with possible negative effects on the protected habitats or species needs a license. Especially licenses for agricultural activities are needed, as husbandry is the main contributor to NH_3 emission. As N deposition exceeds the CL of at least one habitat in all Flemish Habitats Directive areas, the Flemish government also agreed to implement a program for the reduction of N emissions (Dutch: 'Programmatische Aanpak Stikstof', PAS). In the first phase, a consistent tool to evaluate polluting activities will be developed. In a second phase (until January 2019), reduction targets per protected area and per sector (agriculture, industry, traffic) will be fixed. In the final phase, specific measures reducing N emission and a restoration management of the protected areas will be implemented (www.natura2000.vlaanderen.be).

1.3. Nitrogen deposition on forests

Atmospheric deposition to forests has been monitored with sampling and analyses of bulk precipitation and throughfall at several hundred plots for more than 15 years, within the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests). The overall decreasing trend for inorganic N in throughfall was about 2 % during 2000 - 2010 in Level II monitoring plots[1], with the strongest decreasing trends observed in western central Europe in regions where deposition fluxes are highest (Waldner et al., 2014). In unpolluted temperate forest ecosystems an almost closed internal N cycle between plants, microbes, and soil organic matter (SOM) is assumed, with litter production, decomposition, mineralization, immobilization and plant uptake as the main processes involved (Gundersen et al., 2006, Fig. 1.6). As a consequence, decomposition and mineralization are limiting plant and microbial N uptake in undisturbed forests. However, increased N input via atmospheric pollution may disrupt this internal cycle.

The main N compounds polluting the atmosphere are NH_3, its reactive form ammonium (NH_4^+) and NO_x, originating from agriculture, industry and road traffic (Ferm, 1998). Nitrogen can be deposited on the forest canopy in dissolved forms in precipitation droplets (wet deposition) or directly deposited from the atmosphere as gaseous forms and particles, i.e. dry deposition (Harrison et al., 2000). Occult deposition, the deposition via cloud and fog water, is considered negligible in lowland forests compared to wet and dry deposition (Vermeulen et al., 1997).

[1] Level II monitoring plots (around 800) are intensively surveyed to understand complex ecosystem processes, with measurements of forest condition, soil and foliar analyses and additional measurements of tree growth, stand structure, lichens, ground vegetation, litter fall, atmospheric deposition, soil solution chemistry, ambient air quality, meteorology and phenology. Level I monitoring plots are assessed annually on forest condition, and soil and foliar analyses based on a 16 x 16 km grid net covering around 6000 plots in Europe. (http://icp-forests.net/)

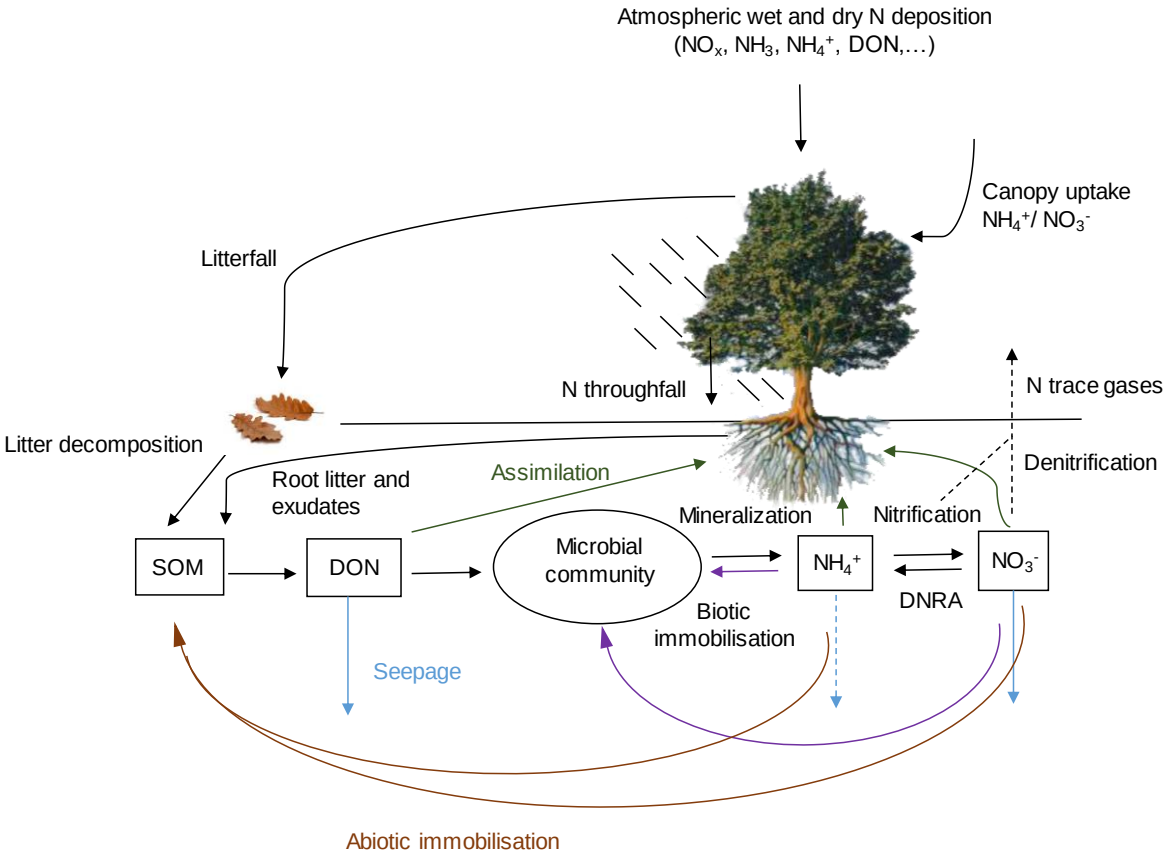

Fig. 1.6: Simplified model of the N cycle in forests (partly adopted from Davidson et al., 2003, Guerrieri et al., 2015, Gundersen et al., 2006, Rennenberg et al., 1998, Schimel and Bennett, 2004). SOM stands for soil organic matter, DON for dissolved organic nitrogen, and DNRA for dissimilatory reduction of nitrate (NO_3^-) to ammonium (NH_4^+). Black dotted arrows indicate gaseous N forms, blue dotted arrow indicates minor seepage of NH_4^+, compared to DON and NO_3^-. Green arrows indicate N assimilation by plants, brown arrows indicate abiotic immobilisation of NH_4^+ and NO_3^- into SOM and purple arrows refer to biotic immobilisation, i.e. the uptake of NH_4^+ and NO_3^- by the microbial community.

1.3.1. Soil processes

Depolymerization of peptides, proteins and other components of detritus and litter produces free amino acids (FAA, Mooshammer et al., 2012). Mineralization transforms organic N to inorganic N and is performed by a whole array of microorganisms (Jansson and Persson, 1982; Fontaine and Barrot, 2005). Gross N mineralization depends on the FAA production rate as FAA mineralization is the main pathway of NH_4^+ production (Geisseler et al., 2012). Next to inorganic N (NO_3^- and NH_4^+), plants are also able to take up organic N, especially FAA, via symbiosis with mycorrhiza (Näsholm et al., 2009). Nitrification converts the less mobile NH_4^+ to mobile NO_3^- that is easily leached out of the soil profile. Moreover, during

nitrification protons (H^+) are produced, acidifying the soil via the following reactions (Eq. 1.1 and 1.2)

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad \text{(Eq. 1.1)}$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad \text{(Eq. 1.2)}$$

Nitrification is predominantly carried out by autotrophic bacteria and Archaea, but to a small extent also by heterotrophic bacteria and fungi (Margesin et al., 2014; Wessén et al., 2011). Equations 1.1 and 1.2 are carried out by different microorganisms. The first step converts NH_4^+ to nitrite (NO_2^-) and is performed by bacteria (genera *Nitrosomonas* and *Nitrosococcus*) and Archaea (Crenarchaeota phylum). The second step, converting NO_2^- to nitrate (NO_3^-) is driven by bacteria of the genus *Nitrobacter* (Gomez, 2014).

Nitrogen can leave the forest soil via leaching (as NH_4^+, NO_3^- or dissolved organic N) or in gaseous forms, i.e. as nitric oxide (NO) and nitrous oxide (N_2O) formed as by-products during nitrification and denitrification, as nitrogen gas (N_2) which is the end-product of denitrification or as NH_3, which can be volatized from alkaline soils. Denitrification occurs where oxygen is depleted and NO_3^- is used as a substitute terminal electron acceptor (Eq. 1.3) (Smith, 2012).

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \quad \text{(Eq. 1.3)}$$

Complete denitrification consists of four reaction steps (Eq. 1.4) in which NO_3^- is reduced into N_2 by the metallo-enzymes nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Philippot, 2002)

$$NO_3^- \xrightarrow[Nar]{} NO_2^- \xrightarrow[Nir]{} NO \xrightarrow[Nor]{} N_2O \xrightarrow[Nos]{} N_2 \quad \text{(Eq. 1.4)}$$

Denitrifiers are a phylogenetic heterogeneous group of microbes, comprising bacteria, Archaea and fungi (Butterbach-Bahl et al., 2013). For instance, *nirK* and *nirS* genes, encoding the key enzyme nitrite reductase, have been assigned to unrelated affiliations (Philippot, 2002).

1.3.2. Effects of increased N deposition

Since forests are generally N limited, N deposition may have a fertilizing effect by increasing forest growth and litter fall (Mol Dijkstra et al., 2009). A range of studies has shown positive forest growth and C sequestration responses under low to moderate N additions (e.g. Franklin et al., 2003). However, high or long-term N addition may negatively impact forest ecosystems by affecting microbial biomass and activity and therefore alter rates of important microbial processes such as net N mineralization and nitrification (Wallenstein et al., 2006). Bahr et al. (2013) showed that moderate N deposition levels were sufficient to reduce growth of ectomycorrhizal mycelia and increase N leaching. Several studies have shown that increased atmospheric N deposition stimulates N_2O emission from forest soils (e.g. Butterbach-Bahl et al., 2002). Nitrous oxide and NO are important greenhouse gases (GHGs), since N_2O has a long residence time and leads to ozone (O_3) destruction in the stratosphere, while NO will lead to ozone production in the troposphere (IPCC, 2013). However, due to its reactivity, part of the emitted NO will react with O_3 to form NO_2, which can again be deposited on or taken up by the forest canopy (Dorsey et al., 2004).

Nitrogen saturation will occur when "the N availability is in excess of total plant and microbial nutritional demand" (Aber et al., 1989) and is indicated by elevated N losses (via leaching and gaseous emission). Beside eutrophication, other harmful effects of increased N inputs include soil acidification, i.e. increasing loss of exchangeable cations and mobilizing aluminum (Al^{3+}) and other potentially toxic metals (Mulder et al., 1987; Wilpert et al., 2000), pollution of groundwater reserves (Koopmans et al., 1995), increased susceptibility of insect attack (Pitman et al., 2010) and species loss (De Schrijver et al., 2011). For instance, De Schrijver et al. (2000) measured NO_3^- concentrations in the soil solution under a pine forest in Flanders exceeding the CL for drinking water (50 mg l^{-1}, WHO, 1985b). Bobbink et al. (2010) showed that N accumulation is the main driver of changes in species composition across ecosystem types by altering competitive interactions, favouring nitrophilic species and hereby decreasing species richness.

However, Wuyts et al. (2011) found higher N deposition but lower inorganic N leaching at a depth of 90 cm in the first 10 to 20 m of the forest edge of oak, birch and pine monocultures in Flanders. As such, forest edges challenge the current N-saturation paradigm that, in N-saturated forests, high N deposition is generally associated with increased inorganic N leaching, providing the incentive for conducting this study.

1.4. Characteristics of forest edges

A forest edge can be viewed as a transition zone with functional and structural gradients between the forest and the adjacent landscape (Schmidt et al., 2017). Forest edges differ substantially from forest interior zones, where we can distinguish primary effects, e.g., on microclimate and fluxes of nutrients (Weathers et al., 2001), and secondary effects or ecosystem responses, e.g., effects on forest structure and biodiversity (Harper et al., 2005; Broadbent et al., 2008). Solar radiation is a key factor in modifying the microclimate at these transition zones (Schmidt et al., 2017). Dignan and Bren (2003) measured a rapid decrease in radiation (wavelengths of 250-3000 nm) that nearly vanished within 100 m from the forest edge. Furthermore, wind velocity is higher in transition zones, increasing conductivity of heat and gases and consequently transpiration rates (Cienciala et al., 2002). Due to increased solar radiation, higher wind velocity and higher evapotranspiration rates, forest edges often have higher soil temperatures and lower soil and litter moisture content (Herbst et al., 2007; Marchand and Houle, 2006). Riutta et al. (2012) measured higher litter decomposition rates in the forest interior compared to the forest edge, due to moisture limitation at the drier forest edge. Crockatt and Bebber (2015) observed an increased decomposition rate of decaying wood with distance from the edge, correlated with increasing humidity and moisture content of the decaying wood. Mean air temperature decreased slightly with distance from the edge in their study. The magnitude of the edge effect on microclimate depends among others on orientation, forest structure and management, as Matlack (1993) measured strong microclimatic gradients in recently created edges facing south, west and east. However, these gradients receded over time as forest edges developed a continuous side canopy.

In general, forest edges are steep transitions of vegetation height, which drastically disrupt air flow (Wuyts, 2009b). Due to obstruction of the wind profile, local advection and turbulent exchange cause an increased atmospheric deposition at forest edges (Draaijers, 1993). The edge effect on atmospheric deposition spans from 15 to more than 100 m from the edge to the forest's interior and causes an up to five-fold increase in throughfall deposition compared to the forest interior (De Schrijver et al., 2007, Wuyts et al., 2008a, 2008b, see Fig. 1.7).

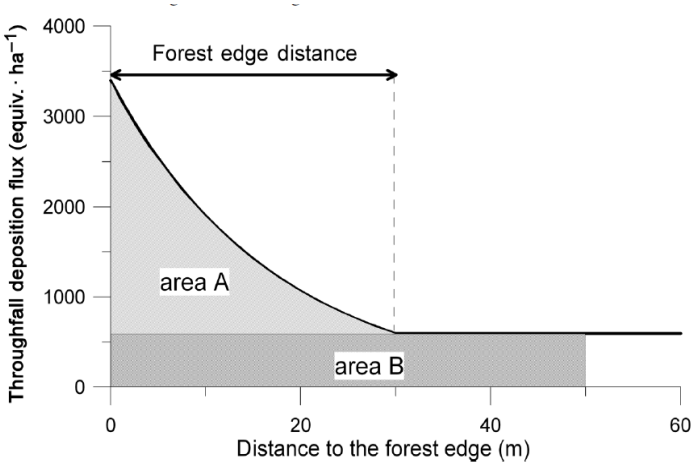

Fig 1.7: Forest edge effect on the throughfall deposition flux of atmospheric cations and potentially acidifying ions with a constant interior level (area B) and increased deposition flux due to the presence of the forest edge (area A). The "forest edge distance" is a distance that varies with tree species, edge structure, forest management and the ion considered (Wuyts et al., 2009b).

The magnitude and depth of the edge effect depend on tree species, edge structure, forest management and is ion-specific (Draaijers, 1993; De Schrijver et al., 1998; Devlaeminck et al., 2005; Wuyts et al., 2008a, 2008b, 2008c, 2009b). Devlaeminck et al. (2005) measured an elevated deposition of Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} at the forest edge (0 – 50 m) of a beech forest in Flanders. Hansen et al. (2007) and Wuyts et al. (2008b) found pronounced gradients in N deposition at the edges of Norway spruce (0 – 25 m) and Corsican pine forests (0 – 15 m), whereas the gradients were less pronounced at the edge of oak and silver birch forests. De Schrijver et al. (2000) attributed the higher N deposition in coniferous forests compared to deciduous forests to the higher Leaf Area Index (LAI), their evergreen character and the higher collecting efficiency of needles compared to leaves.

Furthermore, Wuyts et al. (2009b) showed that gradual forest edges can mitigate edge effects on throughfall deposition if their size and shape are well considered. When incorporating the atmospheric deposition on the gradual edge itself, throughfall fluxes were on average 60 % lower in winter and 74 % lower in summer compared to a steep forest edge, without a herbaceous fringe, shrub belt or forest mantle. Forest edges can act as local biodiversity hotspots, as they harbour both forest species and species from the adjacent landscape (Magura, 2002; Ohwaki et al., 2007, 2015). Due to their role as transition zones, forest edges can act as refuge habitats or stepping stone biotopes. Wermelinger et al. (2007) found a higher insect species richness in gradual edges which provide more resources such as food and shelter compared to steep edges. Bird communities are also strongly affected

by forest patch size and the edge effect (Banks-Leite et al., 2010). Batáry et al. (2014) observed a higher abundance of tree and shrub breeders at edges of the Hainich National Park in Germany. However, the higher bird density at forest edges might lead to higher nest predation and parasitism rates (Batáry and Báldi, 2004).

1.5. Forests as nitrogen and carbon sinks/sources

Nitrogen and C cycles in the forest soil are tightly related as N plays an important role in the C balance and vice versa (Bonan, 2008). As mentioned in the sections above, fragmentation leads to altered solar radiation, temperature, wind, microclimate, and N deposition, all of which have the potential to impact forest N and C cycling either directly or indirectly. At edges, higher N deposition and irradiation could increase C sequestration by increased growth and increased accumulation of soil organic matter (SOM) (Janssens et al., 2010), but higher soil temperatures could increase respiratory losses of microbes. On the other hand, N sequestration in the soil can be enhanced if the supply of labile C from litter turnover to soil microbes is not limited (Huntington, 2005). De Vries et al. (2009) reviewed the impact of N deposition on C sequestration in temperate forests and obtained an average response of 20 to 40 kg C/kg N in aboveground biomass and soil. However, these results may not apply to forests in high N deposition areas. With increasing N-enrichment, N immobilization will decrease and consequently less C will be sequestered per unit of N deposited (De Vries et al., 2009) as soil and microbial biomass C and N concentrations are well-constrained at the global scale via consistent stoichiometry (Cleveland and Liptzin, 2007). Furthermore, Gundersen et al. (2006) showed that the forest floor C/N ratio is a good indicator of the N status of a forest, where NO_3^- leaching has been found beneath a threshold C/N ratio of 25 and N retention above this threshold, emphasizing the importance of soil C content on N retention.

Forests play an important role in climate change mitigation as they are recognized as major C sinks by removing carbon dioxide (CO_2) from the atmosphere (IPCC, 2013). However, massive deforestation has been taking place, reducing storage capacity for C and also releasing additional C into the atmosphere through decay and burning (Ryan et al., 2010). Recent global C analyses have estimated a net global forest C sink of 1.1 ± 0.8 Pg C yr^{-1}, (Pan et al., 2011). De Vos et al. (2015) estimated soil organic C stocks of the forest floor and mineral soil at the European scale and obtained 3.5 – 3.9 Gt C in forest floors and 21.4 – 22.7 Gt C in mineral soil down to 1 m. Vande Walle et al. (2005) estimated the C stock of above- and belowground forest biomass in Flanders and obtained a mean C stock of 85.2

Mg ha^{-1}. Next to CO_2, forest soils can also take up atmospheric methane (CH_4) and are now recognised as a major CH_4 sink in terrestrial ecosystems (Dutaur and Verchot, 2007). Methane has the second highest radiative forcing of all GHGs and its atmospheric concentration has increased by 150 % since pre-industrial times (IPCC, 2013). Major biogenic sources of CH_4 are rice agriculture, ruminants and wetlands (> 50 Tg yr^{-1}), while landfills, coal mines, biomass burning, urban areas, sewage disposal, lakes, oceans and tundra are considered minor sources of CH_4 (Khalil, 2013).

In the long term, the soil is the key N sink in temperate forest ecosystems, and N sequestration is believed to become an increasingly important ecosystem service (Castellano et al., 2012). However, we do not know how and to what extent forest edges affect N and C sequestration and cycling in general. Because of the uncertainty in the forest sink strength and the possible change in magnitude over time, constraining these estimates is important to support future climate mitigation actions. However, the actual estimated sink strength of forests does not differentiate between forest edge and interior. Therefore, the importance of incorporating forest edges when monitoring N and C storage on a landscape scale should be investigated. Moreover, there is a need for studies that generate knowledge on how global environmental changes (including higher N deposition, higher temperatures, and land-use changes) affect ecosystem N cycling to take into account the role of N in the C cycle and climate feedback mechanisms (Bonan, 2008).

1.6. Aims and schematic overview of the thesis

Forest edges are increasingly important landscape features worldwide, but they have largely been ignored in assessments of forest ecosystem functioning. Ruffell and Didham (2016) stated that predicting and managing edge effects requires an understanding of the mechanisms that drive them. Wuyts et al. (2011) made some counterintuitive observations at forest edges, as they found higher N deposition but lower inorganic N leaching in the first 10 to 20 m of oak, birch and pine monocultures in Flanders. Their study focused on N throughfall and leaching, where no clear hydrological patterns could be distinguished along the edge-to-interior transects. They did not include measurements of mineralization or nitrification rates, microbial biomass and N allocation to trees in their study. To explain the decreased inorganic N leaching at the edge, they hypothesized that increased N retention, gaseous N emissions and dissolved organic N (DON) leaching in the soil were the main processes involved in the altered N cycle at the N enriched forest edge. Therefore, the overall aim of this work is to scrutinize the effect of edge proximity on N and C cycling and

sequestration in temperate forest ecosystems. More specifically, this thesis aims to answer two main research questions:

1) Are N and C stocks and sequestration affected by edge proximity?

2) Which processes of the forest N cycle differ between forest edge and interior?

A schematic overview of the thesis is given in Fig. 1.8. The experiments were executed in six temperate forests in Flanders and Denmark growing on acid, sandy quartz-dominated Podzols with a low base saturation, from which data on N throughfall and leaching were available from previous research (Wuyts et al., 2008a, 2008b, 2009a, 2011; Ginzburg, 2014). A schematic representation of the soil profile can be found in Fig. A-I. The L layer comprises the Oi horizon and the FH layer comprises Oe and Oa horizons. In the studied forests, a thin A horizon covered the eluvial E horizon. The B horizon was typically found below a depth of 35 cm. As soil pH was low (pH-H_2O ≤ 4.5), soils would have reached the Al^{3+} buffering range (Bowman et al., 2008). An overview of the Al^{3+} concentration within the mineral topsoil (0 – 5 cm, 5 -10 cm and 10 – 30 cm) of the Belgian stands can be found in Table A-I. The selected forest edges comprised tree species relevant for their respective region. Four forest stands are situated in Belgium: a pedunculate oak (*Quercus robur* L.) forest in Wortegem, West Flanders (Qr1), a second pedunculate oak forest in Ravels, Antwerp (Qr2), an Austrian pine (*Pinus nigra* ssp. *nigra* Arnold) forest in Zedelgem, West Flanders (Pn1), and a Corsican pine (*P. nigra* ssp. *laricio* Maire) forest in Ravels, Antwerp (Pn2). Two spruce forest stands are situated in Denmark on the peninsula of Jutland: one in Sonder Omme (Ps, *Picea sitchensis* (Bong.) Carr., central Jutland) and another in Lemvig (Pa, *Picea abies* (L.) Karst, western Jutland). All the forest edges bordered arable lands dominated by intensive livestock production (pig, poultry and cattle farms) and have experienced several decades of elevated N deposition. Mean yearly NH_3 concentrations were between 6.01 – 7.0 µg m^{-3} for Qr1 and Pn1, between 7.01 – 8.5 µg m^{-3} for Qr2 and Pn2 (www.vmm.be), between 1.0 – 1.5 µg m^{-3} in Ps and between 1.5 and 2.0 µg m^{-3} in Pa (Geels et al., 2012). In Belgium, mean NH_3 concentrations were measured from June 2015 until June 2016, while Danish mean NH_3 concentrations apply for the year 2007. In each forest, an edge-to-interior transect was laid out and experiments were conducted at the edge (0 – 8 m), at 16, 64 and 128 m. An overview of the stand and physicochemical characteristics can be found in Table 1.1. Previous land use (extracted from topographic maps) was in all cases heathland until afforestation in last century. The considered forest edges are facing the locally prevailing wind direction (west to southwest). Mean wind speed was 3.7 m s^{-1} in Qr1 and Pn1, 2.6 m s^{-1} in Qr2 and Pn2 and 6 m s^{-1} in Ps and Pa.

Table 1.1: Characteristics of the six selected forest edges. Values between brackets represent standard deviations.

Stand characteristics	Oak (*Q. robur*)		Pine (*P. nigra*)		Spruce (*P. sitchensis*)	(*P.abies*)
Code	**Qr1**	**Qr2**	**Pn1**	**Pn2**	**Ps**	**Pa**
Region	Belgium (West Flanders)	Belgium (Antwerp)	Belgium (West Flanders)	Belgium (Antwerp)	Denmark (central Jutland)	Denmark (western Jutland)
Coordinates	50°52'08"N 03°27'59"E	51°24'44"N 05°02'45"E	51°08'26"N 03°06'36"E	51°26'37"N 05°05'14"E	55°51'04"N 08°55'56"E	56°29'54"N 08°19'16"E
Age (year)§	98	76	73	51	63	68
Orientation§	SW	SW	SW	SW	W	W
Stem density (n trees ha^{-1})[a]	117	130	109	504	705	688
Basal area (m^2 ha^{-1})[b]	21.5	22.0	11.9	36.4	75.9	45.0
Average height (m)	31.9 (4.0)	24.8 (5.8)	24.9 (3.4)	22.6 (4.9)	30.7 (4.2)	20.5 (2.4)
Soil classification	PZ[c]	PZ	PZ	PZ	PZ	PZ
Physicochemical characteristics						
N TF deposition edge[d] (kg ha^{-1} $year^{-1}$)§	18.5	30.2	60.7	51.5	27.0	43.0
N TF deposition interior[e] (kg ha^{-1} $year^{-1}$)§	17.5	23.4	31.0	39.6	17.0	25.0
pH (KCl) FH[f] layer	3.5	3.0	3.0	3.0	2.8	3.6
pH (KCl) 0-5 cm mineral soil§	3.4	2.9	2.9	2.9	2.9	3.4
C/N ratio FH layer§	18.1 (1.9)	17.8 (1.1)	22.9 (1.5)	25.5 (2.0)	29.3 (1.0)	28.2 (2.5)
C/N ratio 0-5 cm mineral soil	17.6 (1.4)	19.0 (1.8)	22.1 (2.8)	26.9 (4.4)	19.3 (2.5)	22.2 (3.8)

§ Data obtained from Wuyts et al. (2008b) for the Belgian stands (Qr1, Qr2, Pn1 and Pn2) and from Ginzburg (2014) for the Danish stand Pa. For Pa, pH (H_2O) was converted to pH (KCl) according to the European Soil Data Center (ESDAC, esdac.jrc.ec.europa.eu). For Ps, fresh FH and mineral soil (0-5 cm) samples were analyzed for pH (KCl). pH values were measured at the forest interior.

[a] Stem density averaged over the whole transect, obtained by dividing the number of trees by the inventoried area.

[b] Basal area averaged over the whole transect, obtained by dividing the total basal area by the inventoried area. Basal area of each tree was obtained via the inventory of the diameters at breast height ($A = \pi r^2$) and summed to obtain total basal area.

[c] PZ = Podzol: soils characterized by subsoil accumulation of humus and/or oxides (WRB, 2014).

[d] Nitrogen throughfall (TF) deposition values for the first 2 meters of the forest edges.

[e] Nitrogen throughfall (TF) deposition values for the 64 and 128 m plots.

[f] FH = fermentation and humus layer of the ectorganic horizon.

Relative humidity was in all stands between 80 and 85 %. The mean annual air temperature in Belgium is 10.5°C and 7.4°C on the peninsula of Jutland (Denmark). Mean annual precipitation in Belgium is 800 mm and 900 mm in Jutland, Denmark (data on mean wind speed, mean relative humidity, mean air temperature and mean annual precipitation were obtained from the Royal Meteorological Institute and from the Danish Meteorological Institute respectively for the Belgian and Danish forests, 1981 - 2010). The understory vegetation is composed of ferns (*Dryopteris dilatata* and *Dryopteris carthusiana*) and grasses (*Molinea caerulea* and *Holcus* sp.) in the pine stands and in the edge of the spruce stands. The understory vegetation in the edges of the oak stands is characterized by brambles (*Rubus fruticosus* agg.).

To solve the two research questions, N and C stocks of above- and belowground forest compartments and soil were calculated along the edge-to-interior transects. This is presented in **Chapter 2**, together with an estimate of the soil C sequestration in forest edge and interior. In the following chapters, different pathways of the N cycle (Fig. 1.6) in forest soils were investigated.

In **Chapter 3**, fluxes of N and C trace gases (NO, N_2O and CH_4) were measured at the forest edge and interior via an automated system of static and dynamic measuring chambers, in collaboration with the Karlsruhe Institute of Technology (KIT).

Next, the microbial community was mapped via the extraction of phospholipid fatty acids (PLFA) along edge-to-interior transects and amino sugars (AS) in forest edge and interior. Gross nitrogen mineralization, nitrification and immobilization rates were obtained via an *in situ* ^{15}N pool dilution technique in the forest edge and interior and linked to the microbial community structure. Furthermore, we assessed ^{15}N recovery in simulated throughfall via the ^{15}N tracing method in the edge and interior as a proxy for the long-term dynamics of the N cycle. The results of these important components of the N cycle are presented in **Chapter 4**.

In **Chapter 5**, we present the results of litter decomposition along edge-to-interior transects, monitored during 18 months. Secondly, litter of edge and interior was interchanged to focus on the effect of the microclimate and substrate quality during decomposition. Thirdly, litter of the forest interior was placed in Open Top Chambers (OTC), which create edge conditions (warmer) in the forest interior in the absence of the edge soil decomposer community. These

experiments allowed us to further elucidate the underlying mechanisms of the edge effect on litter decomposition.

Chapter 6 links the results of the previous chapters into a general discussion, finalizing with some concluding remarks and future research. The processes involved in the altered N cycle at the edge are summarized in the conceptual figure of the N cycle in forests (see Fig. 6.1).

This thesis was conducted in six temperate forest edges, bordering agricultural activities and oriented in the prevailing wind direction to insure maximal N deposition. In this way, the edge effect could be studied under a 'worst case scenario'. Moreover, these forest stands were already extensively studied prior to this research, ensuring the availability of data on N throughfall deposition, N leaching, forest floor and mineral soil C/N ratios and soil acidity. Unfortunately, a severe storm hit one of the Danish forests (Pa) in winter 2014 and experiments ceased at this site. The equipment used in Chapter 3 (the automatic measuring chambers for N and C trace gases) was expensive and involved months of preparation to acquire all necessary permits from the Federal Agency of Nuclear Control (FANC) of Belgium. Therefore, it was only conducted in one oak and one pine forest in Flanders (Qr2 and Pn2). Due to the limited length of the tubing, the interior position was fixed at 64 m from the edge in this experiment. The stable ^{15}N isotope, used to measure mineralization and nitrification rates and N recovery in Chapter 4, is also an expensive tool and these experiments were therefore restricted to one forest of each forest type (Qr2, Pn2 and Ps) and to forest edge versus interior (64 m). In Chapter 5, the litter decomposition data of the spruce forest (Ps) were omitted from the analysis as the remaining litter weight in the litterbags was biased due to the weight of freshly fallen needles.

Research question 1: Are N and C stocks and sequestration affected by edge proximity?	*Research question 2:* Which processes of the N cycle differ between forest edge and interior?
Chapter 2 Edge effect on N stocks, C stocks and sequestration	**Chapter 3** Edge effect on fluxes of N and C trace gases (NO, N_2O, CH_4)
	Chapter 4 Edge effect on microbial community and N cycling
	Chapter 5 Edge effect on litter decomposition and nutrient release

Chapter 6 General discussion (linking **Chapter 2, 3, 4** and **5**) and conclusions

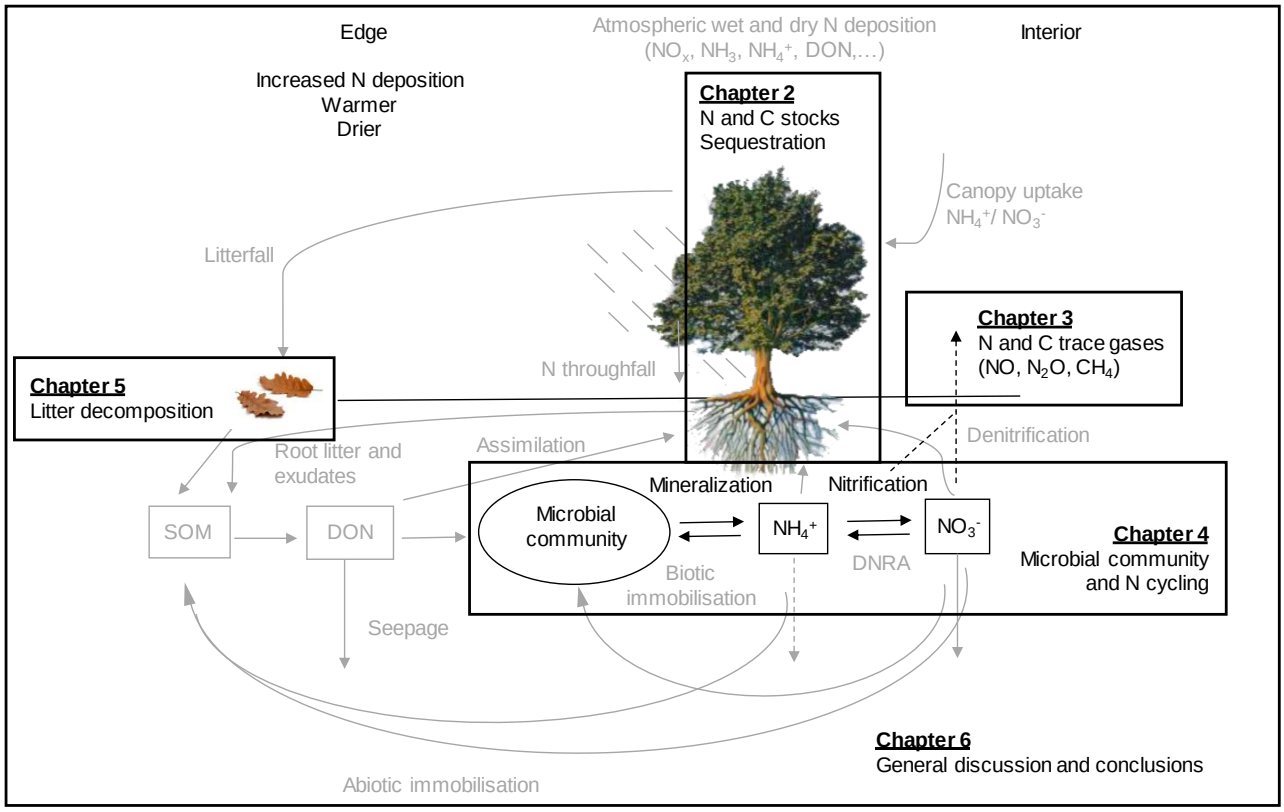

Fig. 1.8: Schematic overview of the thesis

2. Edge effect on N stocks, C stocks and sequestration

After: Remy E., Wuyts K., Boeckx P., Ginzburg S., Gundersen P., Demey A., Van Den Bulcke J., Van Acker J., Verheyen K. (2016). Strong gradients in nitrogen and carbon stocks at temperate forest edges. Forest Ecology and Management 376: 45-58.

Abstract

Due to forest fragmentation, forest edges have become dominant features in landscapes around the world. Forest edges are exposed to a different microclimate in terms of air and soil temperature, light availability, soil moisture and wind speed than the forest interior. Furthermore, forest edges catch more atmospheric deposition, due to obstruction of the wind profile causing advection and turbulent exchange. Coniferous forest types are subjected to higher N deposition due to their higher Leaf Area Index (LAI), evergreen character and higher collecting efficiency of needles compared to leaves. In Europe, highest deposition values coincide with intensive livestock breeding areas, such as northern Belgium. It is still unclear how this elevated atmospheric deposition affects N and C stocks at temperate forest edges. We assessed the N and C stocks of the aboveground (leaves/needles, wood) and belowground (forest floor, coarse and fine roots, mineral soil) forest pools along edge-to-interior transects in six forests, located in Belgium (two oak and two pine stands) and in Denmark (two spruce stands) on acid, sandy Podzols. The total stocks increased towards the forest edge by 30 % for N and 43 % for C, averaged over all forests, within a confidence interval of 95 % (which was in some cases rather wide). The aboveground wood stocks increased by 56 % for N and C, the root stocks by 48 % for N and C and the mineral soil stocks increased by circa 30 % for N and C. Soil C sequestration (calculated via a static N balance based on N throughfall and leaching) increased at the forest edges, being on average 646 and 289 kg ha^{-1} yr^{-1} in the forest edge and interior, respectively. Forest type effects were less prominent than edge effects, with no amplified edge effect on N and C stocks in the coniferous forest types. Nevertheless, our results show the importance of incorporating forest edges when monitoring C storage on a landscape scale. The question arises, however, how much longer such forest edges will continue to accrue additional C when subjected to continuously high atmospheric N deposition.

2.1. Introduction

Central and Western Europe are characterized by small forest remnants resulting from a long-term history of land-use change (Hofmeister et al., 2013). Consequently, forest edges have become important features in the landscape (Harper et al., 2005). Forest edges differ substantially from forest interior zones as shown in the general introduction (§ 1.2). Firstly, edge proximity affects microclimate via air and soil temperature, light availability, soil moisture and wind speed (Marchand and Houle 2006). Secondly, forest edges catch more atmospheric deposition, due to obstruction of the wind profile causing local advection and turbulent exchange (Draaijers, 1993). The edge effect on atmospheric deposition spans 15 to more than 100 m from the edge to the forest's interior and causes an up to five-fold increase in throughfall deposition (De Schrijver et al., 2007). Its magnitude and depth of edge influence depend on tree species, edge structure, forest management and the ion considered (Draaijers, 1993; De Schrijver et al., 1998; Devlaeminck et al., 2005; Wuyts et al. 2008a, 2008b, 2008c, 2009b). Furthermore, in § 1.3 we showed that intensive livestock breeding areas such as Flanders, (northern Belgium) and Jutland (Denmark) still receive high N deposition levels.

The N cycle is closely linked to the C cycle, since C-N interactions constrain the amounts, distributions and turnover rates of C (Agren et al., 1991; Rastetter et al., 1991). For instance, N deposition may increase forest growth and litterfall, hereby augmenting biomass C sequestration (Mol Dijkstra et al., 2009). However, when the photosynthetic capacity of trees is reached, litterfall may remain unchanged or even decrease (Fleischer et al., 2013). On the other hand, increased N deposition reduces fine root biomass and mycorrhizal abundance (Treseder, 2004; Kjoller et al., 2012). Several authors have observed reduced activity of heterotrophic microbes under N addition, slowing down decomposition (DeForest et al., 2004; Ramirez et al., 2012). Forests of mid to high latitudes on the northern hemisphere store most of their C in the organic layer and mineral soil and this stock has a much lower turnover rate than the aboveground C stock (Mol Dijkstra et al., 2009). At present most forests act as sinks for C (De Vries et al., 2006), mitigating CO_2 emission and attenuating global warming (Bonan, 2008). Ziter et al. (2014) point out that the impact of the edge effect on C storage in temperate forests is largely unknown compared to tropical forests. This highlights the need to understand the effects of N deposition on the potential long-term C sequestration in temperate forests.

In this study, the effect of increased N deposition on N and C stocks (aboveground and belowground) at temperate forest edges in an agricultural landscape in Belgium and Denmark was investigated. N and C stocks along edge-to-interior transects were examined in two deciduous and four coniferous monocultures, while controlling for possible confounding factors such as soil type, land-use history, forest age (51 – 98 years) and N deposition. We hypothesized that (i) the increased atmospheric N deposition is associated with increased N and C stocks at the forest edges compared to the forest interiors. Furthermore, due to differences in functional traits between coniferous and deciduous tree species (LAI, evergreen character, collecting efficiency) we expected a forest type effect, namely that (ii) differences in N and C stocks between forest edge and forest interior are more pronounced in the pine and spruce stands compared to the deciduous oak stands. Finally, as the strong link between the N and C cycle has previously been demonstrated, we hypothesized that (iii) there exists an edge effect on soil C sequestration values in association with N deposition.

2.2. Material and methods

2.2.1. Site description

Six forest edges, embedded in an agricultural landscape, were selected for detailed characterization. The latitudinal and longitudinal coordinates of the selected forest edges can be found in Table 1.1, together with an overview of the stand and physicochemical characteristics. Briefly, all forest stands (two oak; Qr1 and Qr2, two pine; Pn1 and Pn2 and two spruce stands Ps and Pa) are even-aged monocultures and grow on acid, quartz-dominated Podzols. Previous land use was in all cases heathland until afforestation in last century. The considered forest edges are facing the locally prevailing wind direction (west to southwest). Mean wind speed was 3.7 m s^{-1} in Qr1 and Pn1, 2.6 m s^{-1} in Qr2 and Pn2 and 6 m s^{-1} in Ps and Pa. Relative humidity was in all stands between 80 and 85 %. The mean annual air temperature in Belgium is 10.5°C and 7.4°C on the peninsula of Jutland (Denmark). Mean annual precipitation in Belgium is 800 mm and 900 mm in Jutland, Denmark (data on mean wind speed, mean relative humidity, mean air temperature and mean annual precipitation were obtained from the Royal Meteorological Institute and from the Danish Meteorological Institute respectively for the Belgian and Danish forests, 1981 - 2010). The understory vegetation is composed of ferns (*Dryopteris dilatata* and *Dryopteris carthusiana*) and grasses (*Molinea caerulea* and *Holcus* sp.) in the pine stands and in the

edge of the spruce stands. The understory vegetation in the edges of the oak stands is characterized by brambles (*Rubus fruticosus* agg.).

2.2.2. Experimental set-up

In each forest one transect was established perpendicular to the forest edge and parallel to the prevailing wind direction. All samples were taken along this transect at four distances: at the edge front (0-2 m), and at 16 m, 64 m and 128 m from the edge. At each distance, two trees were selected from which leaf (or needle), root and wood samples were taken. Soil samples were taken within a 10 m range perpendicular to the transect at each distance. The experimental set-up is shown in Figure 2.1. Samples were taken from July 2013 until May 2014. By selecting temperate forests with the same land-use history and on similar acid sandy soils, we can focus purely on edge and forest type effects.

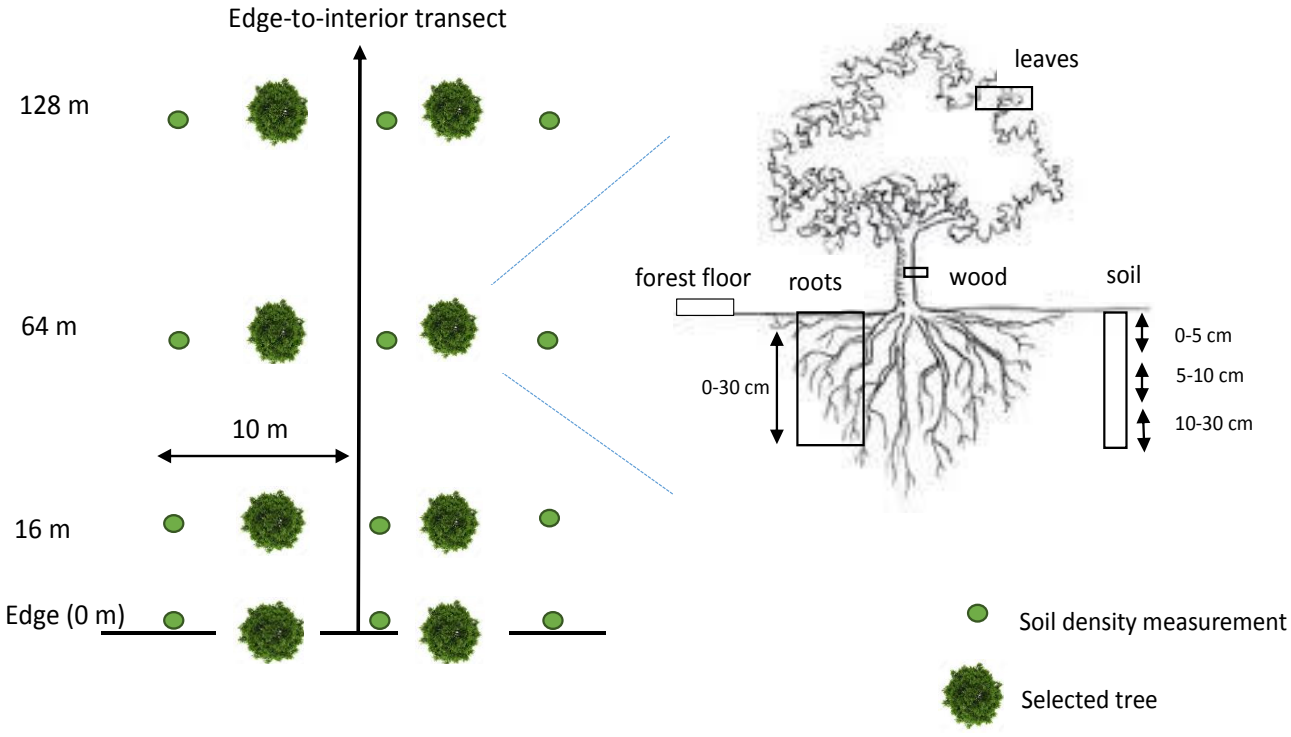

Fig. 2.1: Experimental set-up and sampling overview.

2.2.3. Sampling and analysis

Leaves/needles

Per tree, one branch was selected at a height of at least 10 m and not oriented towards the edge or a gap and sawed after projecting a flexible saw over the branch with a slingshot. From the branch, we took five samples, which consisted of 20 g fully developed, undamaged leaves or of 40 g needles from the previous year's cohort. Leave and needle samples were dried at 65 °C for 2 days and milled (ZM1, Retsch, Germany). Nitrogen and C stocks were calculated based on Eq. (2.1)

$$Stock\ (kg\ ha^{-1}) = (N\ or\ C\ concentration\ (kg\ kg^{-1})\ x\ LAI\ (m^2\ m^{-2})\ x\ LMA\ (kg\ m^{-2}))/ 10^{-4} \quad \text{(Eq. 2.1)}$$

where all variables are mean values per distance. LAI stands for Leaf Area Index (the leaf area per unit ground area) and LMA for Leaf dry Mass per Area (the oven-dry leaf mass divided by the leaf area). The LAI was determined by analyzing six hemispherical photographs per distance with Gap Light Analyzer (GLA version 2.0 1999). The photographs were taken with a fisheye lens (Sigma circular fisheye 8 mm f/4 EX DG), with the following manually adapted camera settings: frame quality JPEG NORM, frame width L (3008 x 2000 pixels), no flash, a shutter time of 1/125 s and an infinite focusing. The aperture was first determined in open field in front of the forests and two stops were subtracted for the use in the forest. Hemispherical photographs were registered in GLA by entering the coordinates of the initial point (north) and the final point (south) of the photograph. When the upper part of the camera is facing north and camera settings are as mentioned above, these coordinates equal 1504 and 2000 (x and y) and 1504 and 0 (x and y), respectively of initial and final point. Next, a threshold pixel value was set visually. The partition of leaves/needles and air on the obtained black and white working image should equal as best as possible the partition of leaves/needles and air on the original color image. Based on these threshold pixel values, the GLA program calculated LAI values. Leaf surface area was measured with LI-3000 Portable Area Meter (LICOR) and needle surface area was estimated from needle length, as described in Wuyts et al. (2011).

Wood

From each selected tree, two wood samples were taken, perpendicular to each other in the horizontal plane, with an increment borer of 30 cm long at a height of 40 cm above the root collar. In the lab they were dried at 40 °C for 2 days to prevent molding. Prior to analysis the wood samples were dried for 24 h at 103 °C. Instead of using mean wood density values

from literature, exact data of the wood density of the six selected forest edges were obtained. Wood samples were x-rayed at intervening steps of 112 µm with a Nanowood micro-CT scanner, installed at the Ghent University Centre for X-ray Tomography (www.ugct.ugent.be). Afterwards the scans were analyzed with Fiji, developed by Schindelin et al. (2012) to extract wood density values. This software program is a variant of ImageJ software, an open tool for the analysis of scientific images (Schneider et al. 2012). An extra set of reference samples were x-rayed to obtain a calibration curve between the wood density obtained via x-ray tomography and the gravimetric wood density. The oven-dry wood density and volume of the reference samples (obtained via the software package Octopus Analysis © 2014) were used to obtain green volume values, based on the volumetric swelling percentages at the fibre saturation point (i.e. a moisture content of 30 %), derived from Jonas et al. (2005) (i.e. 16 %, 14 % and 13 % for respectively oak, pine and spruce). The wood samples were milled (SM2000, Retsch, Germany) and N and C stocks present in the wood were calculated based on Eq. (2.2)

$$Stock\ (Mg\ ha^{-1})\ = (N\ or\ C\ concentration\ (kg\ kg^{-1})\ x\ tree\ volume\ (m^3\ tree^{-1})\ x\ stem\ density\ (n\ trees\ ha^{-1})\ x\ wood\ density\ (kg\ m^{-3}))/10^3 \quad \text{(Eq. 2.2)}$$

where all variables are mean values per distance. Tree volume is calculated with the volume equations of Zianis et al. (2005) (based on mean diameter at breast height, dbh and average height at each distance, Eq. 2.3)

$$Tree\ volume\ (m^3) = (mean\ dbh\ (cm)\ \hat{}\ a\ x\ height\ (m)\ \hat{}\ b\ x \exp(c))\ /1000 \quad \text{(Eq. 2.3)}$$

where a, b and c are 2.00, 0.86 and -2.86; 1.96, 0.89 and -2.17; 1.78, 1.13 and -2.91, respectively for oak, pine and spruce. The height of three trees at each distance was measured with a Vertex (Vertex III, Haglöf, Sweden). For Qr1, Qr2 and Pn1, inventory data are available from previous measurements (Wuyts et al., 2008b) and were updated with re-measurements of the diameters at breast height. The total inventoried area was 7908 m², 3165 m² and 9754 m², respectively in Qr1, Qr2 and Pn1. In Pn2, Ps and Pa, tree inventories were performed on an area expanding 5 m to the left and 5 m to the right of the transect, resulting in a total inventoried area of 1300 m². The number of trees occurring in a zone around each sampling distance (0-10 m, 10-30 m, 30-80 m and 80-130 m from the edge) were counted to obtain stem density per distance. The wood density is the average value at each distance (0, 16, 64 and 128 m).

Roots

Roots were collected at each selected tree by digging out the forest floor and mineral soil in an area of 20 cm x 20 cm to a depth of 30 cm, at a distance of 1 m from the tree trunk. Roots were extracted from this organic and mineral soil layer in the field. In the lab, roots were subsequently rinsed to remove all soil particles. Afterwards they were cut, dried at 65° for two days and milled (ZM1, Retsch, Germany). Coarse and fine roots (< 2 mm) were analyzed separately. Nitrogen and C stocks were calculated based on Eq. (2.4) for the coarse roots and based on Eq. (2.5) for the fine roots.

$$Stock\ (Mg\ ha^{-1}) = (N\ or\ C\ concentration\ (kg\ kg^{-1}) x\ stem\ density\ (n\ trees\ ha^{-1})\ x\ root\ biomass\ (kg\ tree^{-1}))/10^{3} \quad \text{(Eq. 2.4)}$$

$$Stock\ (kg\ ha^{-1}) = N\ or\ C\ concentration\ (kg\ kg^{-1})\ x\ stem\ density\ (n\ trees\ ha^{-1})\ x\ root\ biomass\ (kg\ tree^{-1}) \quad \text{(Eq. 2.5)}$$

Stem density was measured per zone (0-10 m, 10-30 m, 30-80 m and 80-130 m from the edge) as described above in the calculation of the wood stocks and root biomass was calculated via allometric relations, based on mean diameter at breast height per distance for coarse (Eq. 2.6) and fine roots (Eq. 2.7), taken from respectively Jenkins et al. (2003) and Liski et al. (2002).

$$Coarse\ root\ biomass\ (kg\ tree^{-1}) = aboveground\ biomass\ x\ coarse\ root\ ratio$$

$$= \exp(-2.0127 + 2.4342\ x\ ln(dbh))\ x \exp(-1.6911 + (0.816/dh)) \quad \text{(Eq. 2.6)}$$

$$Fine\ root\ biomass\ (kg\ tree^{-1}) = aboveground\ biomass\ x\ stem\ ratio\ x\ fine\ root\ ratio$$

$$= \exp(-2.0127 + 2.4342\ x\ ln(dbh))\ x \exp\left(-0.3065 + (-5.424/dbh)\right)\ x\ 0.02 \quad \text{(Eq. 2.7)}$$

where coarse root ratio is the ratio of the coarse root biomass to total aboveground biomass, stem ratio is the ratio of the stem wood to total aboveground biomass for trees with a diameter at breast height of minimum 2.5 cm, and fine root ratio is the ratio of the fine root biomass to the stem biomass.

Forest floor

The biomass of the fermentation and humus (FH) layer of the ectorganic horizon was determined by collecting all FH material in a square of 0.39 m by 0.39 m (Belgium) or 0.25 m x 0.25 m (Denmark). Three (Belgium) or four (Denmark) FH samples were taken at each distance along the transect (Fig. 2.1). The samples were dried at 65°C during two days,

weighed and milled (ZM1, Retsch, Germany) for further analysis. Nitrogen and C stocks of the forest floor were calculated based on Eq. (2.8)

$$Stock\ (Mg\ ha^{-1}) = N\ or\ C\ concentration\ (kg\ kg^{-1})\ x\ forest\ floor\ mass\ (kg\ m^{-2}) x\ 10 \quad \text{(Eq. 2.8)}$$

Mineral soil

Mineral soil density was measured by means of Kopecky rings at three different depths (0 to 5 cm, 5 to 10 cm and 10 to 30 cm, Fig. 2.1) and at three different locations per distance. After being dried at 105°C for two days and weighed, soil density was calculated based on Eq. (2.9)

$$Soil\ density\ (g\ cm^{-3}) = W_d\ (g)/V_k\ (cm^3) \quad \text{(Eq. 2.9)}$$

where W_d is the weight of the dried soil and V_k is the volume of the Kopecky rings (100 cm^3). This has been done during each season, where the average soil density value was used for the calculation of soil N and C stocks. Soil moisture was determined from the same samples. To measure the N and C concentration, the mineral soil from 0 to 5 cm, from 5 to 10 cm and from 10 to 30 cm was sampled with a soil auger at two locations at each distance along the edge-to-interior transect. The samples were dried for two days at 40°C, sieved over a 2 mm mesh and ground to a size of 0.25 mm (ZM1, Retsch, Germany). Nitrogen and C stocks in soil were calculated based on Eq. (2.10) for the three depths and summed afterwards.

$$Stock\ (Mg\ ha^{-1}) = N\ or\ C\ concentration\ (g/100\ g)\ x\ soil\ density (g\ cm^{-3})\ x\ soil\ depth\ (cm) \quad \text{(Eq. 2.10)}$$

All samples (leaves/needles, wood, forest floor, roots and mineral soil) were kept cool during transport. Nitrogen and C concentrations of all samples were measured by the same CNS elemental analyzer (vario Macro Cube, Elementar, Germany).

2.2.4. Soil carbon sequestration

Soil carbon sequestration (ΔC) was estimated via Eq. (2.11), derived from Gundersen et al. (2006)

$$\Delta C = \Delta N\ x\ (C/N)_{forest\ floor} \quad \text{(Eq. 2.11)}$$

where net soil N immobilisation (ΔN) as an average for a forest rotation can be calculated from a N budget as

$$\Delta\ N = deposition + fixation - harvesting - leaching - denitrification \quad \text{(Eq. 2.12)}$$

given that the budget terms are constant over time. In these static N balance calculations only vertical percolation, i.e. deposition and leaching, was considered. Yearly N throughfall deposition and inorganic N leaching in Qr1, Qr2, Pn1 and Pn2 were obtained from a previous study in the same forest stands and at exactly the same distances from the edge (Wuyts et al., 2008b, 2011). Since then, systematic decreases in inorganic N deposition have been observed in north central Europe (including Flanders and Denmark) (Waldner et al., 2014), and these were comparable for all study sites (Staelens et al., 2012b). In Ps and Pa, yearly N throughfall deposition and soil solution NO_3^- concentrations were obtained as described in Ginzburg (2014). Monthly throughfall volumes were collected by three polyethylene funnels located at increasing distances from the edge (10 m, 20 m, 40 m, 60 m and 100 m) during one (2010 - 2011 for Pa) or two years (2011 - 2013 for Ps). The funnels were located on poles about 1-1.5 m above the ground, while the plastic bottles collecting the water were placed in a plastic cylinder underground to reduce any effects of temperature or light on N processes in the water. Samples were kept cool before NO_3^- and NH_4^+ content in the throughfall water were determined by ion chromatography and flow injection analysis, respectively. The N deposition was calculated by multiplying the N concentration in the sub-sample by the throughfall volume collected in the three funnels divided by the surface area of the funnels. At Ps and Pa, soil solution NO_3^- concentrations were measured by KCl extraction of mineral soil, sampled in spring 2010 and November 2012 using a soil corer (diameter of 3 cm). Annual mean NO_3^- concentrations (mg l^{-1}) were converted to an annual N leaching flux (kg ha^{-1} yr^{-1}) via an empirical relationship, Eq. (2.13), derived by Gundersen et al. (2009)

$$N\ leaching\ (kg\ ha^{-1}\ yr^{-1}) = 2.06\ x\ annual\ mean\ NO_3^-\ concentration\ (mg\ l^{-1}) + 2 \quad \text{(Eq. 2.13)}$$

This relationship was obtained via intensive monitoring of three coniferous and five deciduous stands in Denmark during 3 to 4 years. Monthly N output by seepage water was calculated as the concentration of NO_3^- in the soil water samples from 0.9 m soil depth (collected via porous PTFE suction cups) multiplied with the seepage water flux, modelled with the CoupModel (Jansson et al, 1999). Since biological fixation, harvesting and denitrification contribute little to the N budget on the selected acid, sandy soils these processes were neglected (Akselsson et al., 2007). Estimated yearly N trace gas emission for Qr2 and Pn2 amounted to respectively 0.6 and 0.2 kg N_2O ha^{-1} and 1.5 and 1.3 kg NO_x ha^{-1} (unpublished data). Hence, N losses via denitrification were minimal and omitted from Eq. (2.13). Soil (organic layer and mineral soil) carbon sequestration values were divided by

N throughfall deposition values to obtain the amount of C sequestered per unit (kg) of N deposited, which will be further termed as the C sequestration response to N deposition (C_{resp}). Since data on N allocation to other forest pools lack, we do not attempt to calculate C sequestration for the whole forest ecosystem, but focus on the impact of N deposition on soil C sequestration.

2.2.5. Statistical analyses

All statistical analyses were performed in R (version 3.1.2.), using the lme4 package. We tested if variations in N and C stocks, and in the variables used to calculate N and C stocks, were associated with the examined forest type and distance to the forest edge. We used the following variables as response variables: N and C stock, and N and C concentration of all forest pools, stem density, LAI, LMA, wood density, wood volume, root biomass, forest floor mass, soil density and soil C sequestration. Predictor variables were distance to the forest edge, forest type (oak, pine, spruce), the interaction of distance to the forest edge and forest type and region. Firstly, the need of a linear mixed effect model, including the forest location (six stand locations) as a random factor, was tested for each stock (2 replicates at each distance in each stand). In this way, the non-independence of samples from the same stand has been taken into account. This linear mixed effect model was compared with a linear model, where both models contained distance to the forest edge, forest type (oak, pine, spruce), their interaction and region as predictor variables. The appropriate model was chosen based on the lowest AIC (Akaike Information Criterion) value. When a linear mixed effect model was needed, the Intraclass Correlation Coefficient (ICC) was calculated via Eq. (2.14)

$$ICC = \sigma_0^2/(\sigma_0^2 + \sigma_\epsilon^2) \quad \text{(Eq. 2.14)}$$

where σ_0^2 is the variance of the intercept and σ_ϵ^2 is the variance of the residuals. The ICC indicates how much of the overall variation in the response variable is explained by the hierarchy of the model, i.e. by clustering the data within the different forest stands. The contribution of each predictor variable to the model was tested with one-way analysis of variance (ANOVA) and presented in Table 2.1, 2.2 and 2.3. The relationship between the fitted values and the residuals of each model was checked to ensure normality and homoscedasticity. The x-axis of Figures 2.2, 2.3, 2.4, 2.5 and 2.6 has a logarithmic scale to improve the spread of the data in the observed range of the distance to the forest edge (0 – 128 m).

2.3. Results

2.3.1. Biomass, forest floor and soil properties

On the foliage properties, no significant edge effects were observed although significant interactions with forest type occurred for LAI (Table 2.1). Forest type effects were significant for all foliage properties, with higher N concentration but lower C concentration in oak leaves than pine or spruce needles and higher LAI and LMA in spruce stands (Table 2.1).

From all wood properties, only stem density and wood volume were significantly affected by the distance to the edge, decreasing with distance (Table 2.1). Forest type significantly influenced the wood C concentration, wood density, wood volume and stem density, showing highest C concentration in pine, higher wood volume and stem density in spruce and highest wood density in the oak stands.

In the coarse roots, the root biomass decreased with distance to the edge. Forest type also affected coarse root biomass, being highest in the oak stands. N and C concentration of the coarse roots were only affected by forest type, being lowest in the spruce trees. In the fine roots, the root biomass also decreased with distance to the edge, while the C concentration increased with distance to the edge. Both fine root biomass and C concentration were affected by forest type and were highest in the oak stands.

In the forest floor, the N and C concentration increased with distance to the edge. The forest floor mass differed between forest type (Table 2.1), being highest in the spruce stands. In the mineral soil, the N concentration was higher at the edge than in the interior plots. Soil density increased with distance to the edge (Table 2.1). The C concentration of the mineral soil was also higher at the edge (except for the outliers at the 16 m plots in the spruce stands). Nitrogen and C concentrations were affected by forest type, being higher in the spruce stands.

Table 2.1: Effects of distance to the forest edge and forest type on the variables used to calculate the N and C stocks of the forest pools.

Stand characteristic	Variable	R^2	Distance to forest edge	Forest type		Interaction	ICC
	Stem density (n ha^{-1})	0.76	**< 0.01** ↓	**< 0.001**	s > o,p	0.095	-
Forest pool							
Leaves/needles	N concentration (kg kg^{-1})	0.86	0.166	**< 0.001**	o > p,s	0.286	-
	C concentration* (kg kg^{-1})	0.68	0.349	**< 0.001**	o < p,s	0.080	-
	LAI (m^2 m^{-2})	0.69	0.062	**< 0.001**	s > o,p	**< 0.001** →	-
	LMA (kg m^{-2})	0.99	0.291	**< 0.001**	s > o,p	0.095	-
Wood	N concentration (kg kg^{-1})	0.07	0.194	0.089		0.401	-
	C concentration§ (kg $kg^{-1)}$	0.62	0.827	**< 0.001**	p > o,s	0.366	0.66
	Wood density (kg m^{-3})	0.64	0.337	**< 0.001**	o > p,s	0.150	-
	Wood volume* (m^3 ha^{-1})	0.68	**< 0.01** ↓	**< 0.01**	s > o,p	0.147	-
Coarse roots	N concentration§ (kg kg^{-1})	0.42	0.407	**< 0.001**	s < o,p	0.556	0.51
	C concentration* (kg kg^{-1})	0.38	0.106	**< 0.001**	s < o,p	0.099	-
	Root biomass* (kg $tree^{-1}$)	0.92	**< 0.01** ↓	**< 0.001**	o > p,s	0.059	-
Fine roots	N concentration (kg kg^{-1})	0.01	0.177	0.404		0.401	-
	C concentration (kg kg^{-1})	0.49	**< 0.001** ↑	**< 0.001**	o > p,s	0.118	-
	Root biomass* (kg $tree^{-1}$)	0.93	**< 0.01** ↓	**< 0.001**	o > p,s	**< 0.05**	0.94
Forest floor	N concentration§ (kg kg^{-1})	0.19	**< 0.01** ↑	0.865		**< 0.01** →	0.46
	C concentration§ (kg kg^{-1})	0.22	**< 0.001** ↑	0.063		**< 0.01** ↑	0.60
	Forest floor mass (kg m^{-2})	0.28	0.786	**< 0.001**	s > o,p	0.179	-
Soil	N concentration (kg kg^{-1})	0.16	**< 0.001** ↓	**< 0.001**	s > o,p	0.321	-
	C concentration* (kg kg^{-1})	0.14	**< 0.001** ↓	**< 0.01**	s > o,p	0.214	-
	Soil density§* (g cm^{-3})	0.10	**< 0.001** ↑	0.177		**< 0.001** ↑	0.46

In case of a linear model, no random term is needed, in case of a linear mixed effect model (indicated with §) the forest location was used as a random term. The * indicates a significant effect ($p < 0.05$) of region. R^2 is the coefficient of determination, indicating the proportion of variation explained by the model. The R^2 and p values of the best fitting model (based on the AIC value) are presented. The forest type effect is specified, where o = oak, p = pine and s = spruce. The ICC indicates how much of the overall variation in the response is explained by the random term of the mixed model. Bold values are significant ($p < 0.05$). The arrow indicates if values increase (↑) or decrease (↓) with distance to the edge. When all forest type increase with distance to the edge, the interaction is positive (↑), when all forest type decrease with distance to the edge, the interaction is negative (↓) and when the interaction term differs between forest type (meaning that for some forest type values increase with distance to the edge, while they decrease for other forest type), the interaction is neutral (→).

2.3.2. Nitrogen stocks

The amount of N stored in leaves and needles ranged from 12 kg ha^{-1} to 53 kg ha^{-1} (Fig 2.2a). The effect of distance from the edge was not significant, but interacted with the forest type effect (Table 2.2), as the oak and pine forests showed weak decreasing trends and the spruce stands a strong increasing trend (Fig. 2.2a). The N stocks of leaves and needles were 21 % and 38 % higher at the edge (0 m) of the oak and pine stands, while at the spruce stands it was 48 % lower than in the interior (128 m). The N stock in spruce needles was higher than in oak leaves and pine needles, resulting in a significant forest type effect.

The amount of N stored in wood ranged between 0.07 Mg ha^{-1} and 3.6 Mg ha^{-1} (Fig. 2.2b). Distance to the edge and the interaction with forest type were significant (Table 2.2). The N stock at the edge (0 m) was 77 % higher than in the forest interior (128 m) in the oak and pine stands and 28 % higher in the spruce stands, leading to an average increase of 61 % at the edge (with upper and lower 95 % confidence intervals of 5 and 115 %).

The values of the N stock of the coarse roots lay between 0.08 Mg ha^{-1} and 3 Mg ha^{-1} (Fig. 2.2c). Distance to the forest edge was significant (Table 2.2). The N stock at the edge (0 m) was 53 %, 66 % and 24 % higher than in the interior (128 m) respectively for oak, pine and spruce, resulting in an average increase of 44% at the edge (with upper and lower 95 % confidence intervals of 21 and 99 %). The fine roots stored N within the range of 7 kg ha^{-1} to 184 kg ha^{-1} (Fig. 2.2d). Distance to the forest edge and forest type contributed most to the model (Table 2.2). At the edge (0 m), the fine root N stock was 64 %, 70 % and 31 % higher than in the interior (127 m) respectively for oak, pine and spruce, resulting in an average increase of 53 % at the edge (with upper and lower 95 % confidence intervals of 26 and 106 %).

The N stock in the forest floor ranged from 0.1 Mg ha^{-1} to 3 Mg ha^{-1} (Fig. 2.3a). Adding distance to the edge to the model had no significant effect, nor adding forest type (Table 2.2).

The N stock in the mineral soil ranged from 0.7 Mg ha^{-1} to 9 Mg ha^{-1} (Fig. 2.3b). Distance to the forest edge, forest type and their interaction influenced the N stock significantly (Table 2.2). The N stock at the edge (0 m) was 39 %, 43 % and 22 % higher than in the forest interior (128 m) for the oak, pine and spruce stands. Averaged over all forest type, the N stock was 33 % higher at the edge (with upper and lower 95 % confidence intervals of 12 and 54 %).

Table 2.2: Effects of distance to the forest edge and forest type on the N and C stocks of all forest pools.

Forest pool	R^2	Distance to forest edge	Forest type		Interaction	ICC
N stocks						
Leaves/needles	0.79	0.904	**< 0.001**	s > o, p	**< 0.001** →	-
Wood§*	0.49	**< 0.001** ↓	0.312		**< 0.001** ↓	0.46
Coarse roots*	0.50	**< 0.001** ↓	0.196		0.109	-
Fine roots*	0.51	**< 0.001** ↓	**< 0.05**	o > p, s	0.060	-
Forest floor§	0.07	0.140	0.284		0.306	0.40
Soil§	0.28	**< 0.001** ↓	**< 0.05**	s > o, p	**< 0.01** ↓	0.49
Aboveground*	0.65	**< 0.001** ↓	**< 0.001**	s > o, p	**< 0.05** ↓	-
Belowground§	0.29	**< 0.01** ↓	**< 0.05**	s > o, p	**< 0.05** ↓	0.59
Total§	0.35	**< 0.001** ↓	**< 0.05**	s > o, p	**< 0.001** ↓	0.61
C stocks						
Leaves/needles	0.93	0.249	**< 0.001**	s > o, p	**< 0.001** →	-
Wood§*	0.54	**< 0.001** ↓	0.338		**< 0.001** ↓	0.46
Coarse roots*	0.63	**< 0.001** ↓	**< 0.001**	o > p, s	0.096	-
Fine roots*	0.60	**< 0.001** ↓	**< 0.001**	o > p, s	**< 0.05** ↓	-
Forest floor§	0.12	**< 0.05** ↑	**< 0.05**	s > o, p	0.057	0.63
Soil§	0.26	**< 0.001** ↓	0.848		**< 0.001** ↓	0.35
Aboveground*	0.68	**< 0.001** ↓	**< 0.001**	s > o, p	**< 0.05** ↓	-
Belowground§	0.21	**< 0.001** ↓	0.070		**< 0.01** ↓	0.39
Total§	0.54	**< 0.001** ↓	0.137		**< 0.001** ↓	0.42

In case of a linear model, no random term is needed, in case of a linear mixed effect model (indicated with §) the forest location was used as a random term. The * indicates a significant effect ($p < 0.05$) of region. R^2 is the coefficient of determination, indicating the proportion of variation explained by the model. The R^2 and p values of the best fitting model (based on the AIC value) are presented. The forest type effect is specified, where o = oak, p = pine and s = spruce.The ICC indicates how much of the overall variation in the response is explained by the random term of the mixed model. Bold values are significant ($p < 0.05$). The arrow indicates if values increase (↑) or decrease (↓) with distance to the edge. When all forest type increase with distance to the edge, the interaction is positive (↑), when all forest type decrease with distance to the edge, the interaction is negative (↓) and when the interaction term differs between forest type (meaning that for some forest type values increase with distance to the edge, while they decrease for other forest type), the interaction is neutral (→).

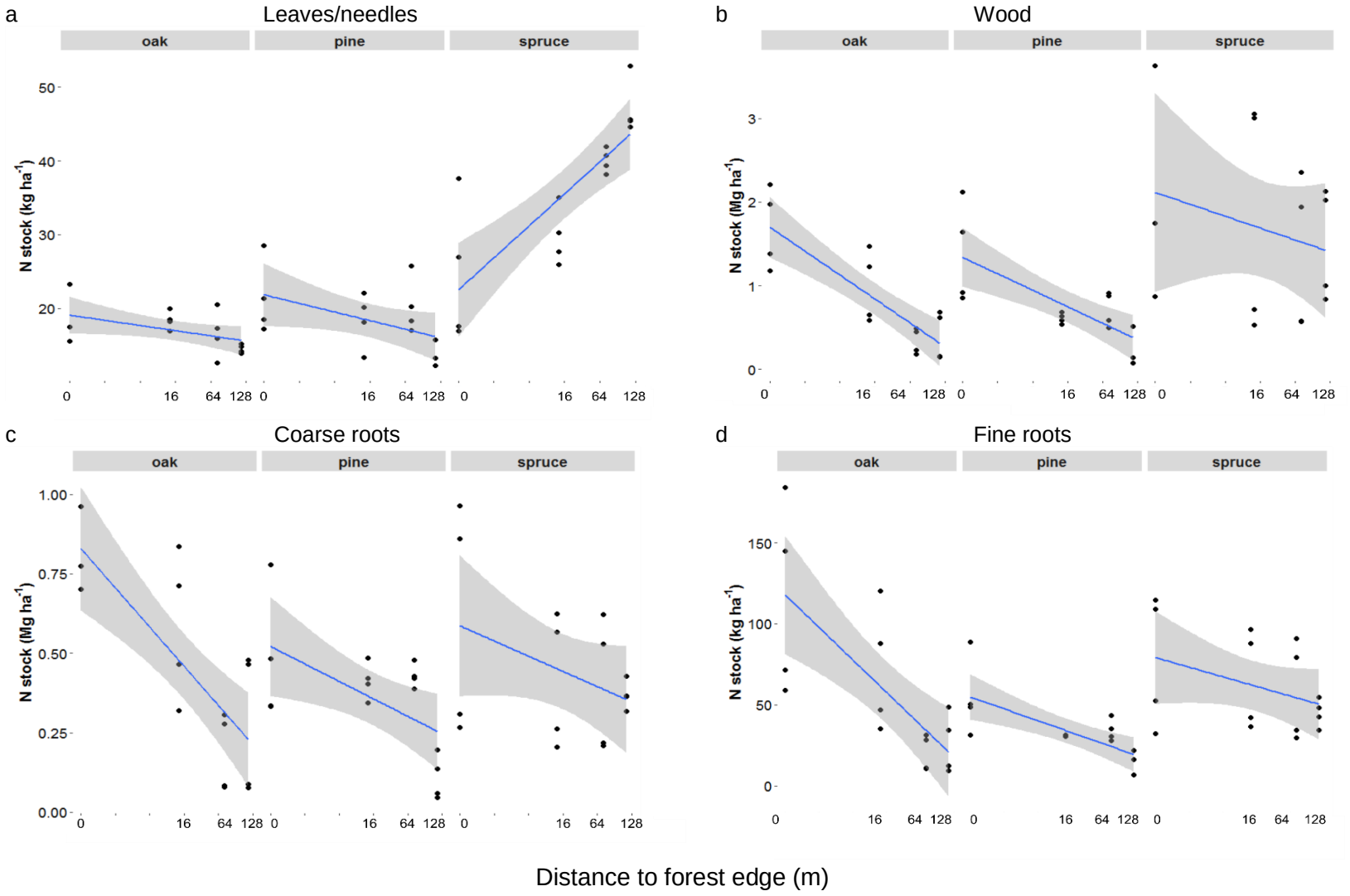

Fig. 2.2: N stock in a) leaves and needles, b) wood, c) coarse roots and d) fine roots (n = 4 at each distance,). Grey shading shows the 95% confidence interval. For readability, x-axes show distance to forest edge in m and not as the logarithm of distance to forest edge. Significant effects of distance to forest edge, forest type and their interaction are presented in Table 2.2.

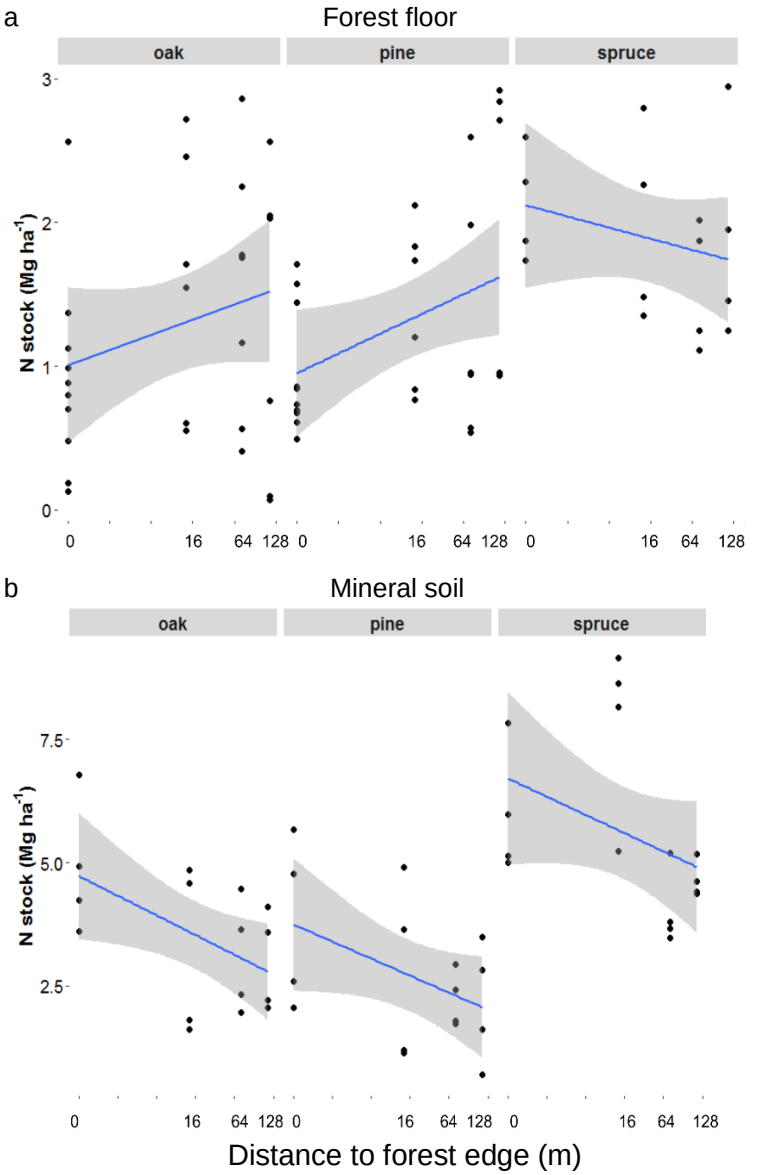

Fig. 2.3: N stock in a) forest floor and b) mineral soil until a depth of 30 cm (n = 4 at each distance, in a) n = 6 in Belgium). Grey shading shows the 95% confidence interval. For readability, x-axes show distance to forest edge in m and not as the logarithm of distance to forest edge. Significant effects of distance to forest edge, forest type and their interaction are presented in Table 2.2.

The total aboveground N stock was significantly affected by distance to the edge, the forest type and their interaction and was, on average, increased by 53 % at the edge (0 m). The total belowground N stock was influenced by distance to the edge, forest type and their interaction and was on average 24% higher at the edge (0 m) compared to the forest interior (128 m). The same applied for the total N stock (Fig. 2.6a), which was on average 29 % higher (with upper and lower 95 % confidence intervals of 11 and 47 %) at the edge (0 m) compared to the forest interior (128 m), and respectively 38 %, 33 % and 20 % for oak, pine and spruce.

2.3.3. Carbon stocks

The C stock of the leaves and needles ranged between 0.3 and 1.9 Mg ha^{-1} (Fig. 2.4a). The variation in this stock was explained by the interaction between distance to the edge and forest type and forest type (Table 2.2). The C stocks of oak leaves and pine needles were respectively 15 % and 32 % higher at the edge (0 m), while the C stock of spruce needles was 56 % lower at the edge compared to the interior (128 m).

The C stock of the wood ranged between 22 and 400 Mg ha^{-1} (Fig. 2.4b) and was affected by distance to the edge and the interaction with forest type (Table 2.2). The C stock at the edge (0 m) was higher than in the interior (128 m), respectively with 75 %, 74 % and 30 %, in the oak, pine and spruce stands resulting in an average increase of 60 % at the edge (with upper and lower 95 % confidence intervals of 9 and 109 %).

The C stock of the coarse roots ranged between 4 and 55 Mg ha^{-1} (Fig. 2.4c) and could be explained by distance to the forest edge and forest type (Table 2.2). The C stock was 60 %, 71 % and 13 % higher at the edge (0 m) of the oak, pine and spruce stands, while the average C stock at the edge was 43 % higher (with upper and lower 95 % confidence intervals of 3 and 109 %) than in the forest interior (128 m). The C stock of the fine roots ranged between 0.2 and 3.7 Mg ha^{-1} (Fig. 2.4d). Again distance to the forest edge and forest type were significant. The fine root C stock was 64 %, 70 % and 10 % higher at the edge (0 m) of the oak, pine and spruce stands, while the average C stock at the edge was 44 % higher (with upper and lower 95 % confidence intervals of 2 and 112 %) than in the forest interior (128 m).

The C stock of the forest floor ranged between 2 and 90 Mg ha^{-1} (Fig. 2.5a) and in this case the linear mixed model comprising of distance to the forest edge and forest type was the most appropriate model (Table 2.2). The stocks were 28 %, 48 % and 4 % lower at the edge (0 m) of the oak, pine and spruce forests than in the forest interior (128 m), resulting in an average stock which was 45 % lower at the edge than in the forest interior. The large variability in the C stock was shown in the boundaries of the 95 % confidence interval, ranging between an increase of 16 % at the edge and a decrease of 70 % at the edge.

The mineral soil C stock ranged between 32 and 202 Mg ha^{-1} (Fig. 2.5b). The best fitting model comprised of distance to the forest edge and the interaction with forest type (Table 2.2). The C stock was respectively 32 %, 30 % and 27 % higher at the edge (0 m) of the oak, pine and spruce stands than in the interior (128 m). When averaged over all species,

the soil C stock was 30 % higher at the edge (with upper and lower 95 % confidence intervals of 24 and 35 %).

The aboveground C stock was affected by distance to the edge, forest type and their interaction (Table 2.2), with an increase at the forest edge (0 m) of 56 % compared to the forest interior (128 m). The belowground C stock was steered by distance to the edge and the interaction with forest type, with an increase at the edge (0 m) of 20% compared to the forest interior (128 m). The total C stock (Fig. 2.6b) was also affected by distance to the edge and the interaction with forest type, with an average increase at the edge of 43 % (with upper and lower 95 % confidence intervals of 0 and 81 %), and respectively 62 %, 50 % and 19 % for oak, pine and spruce.

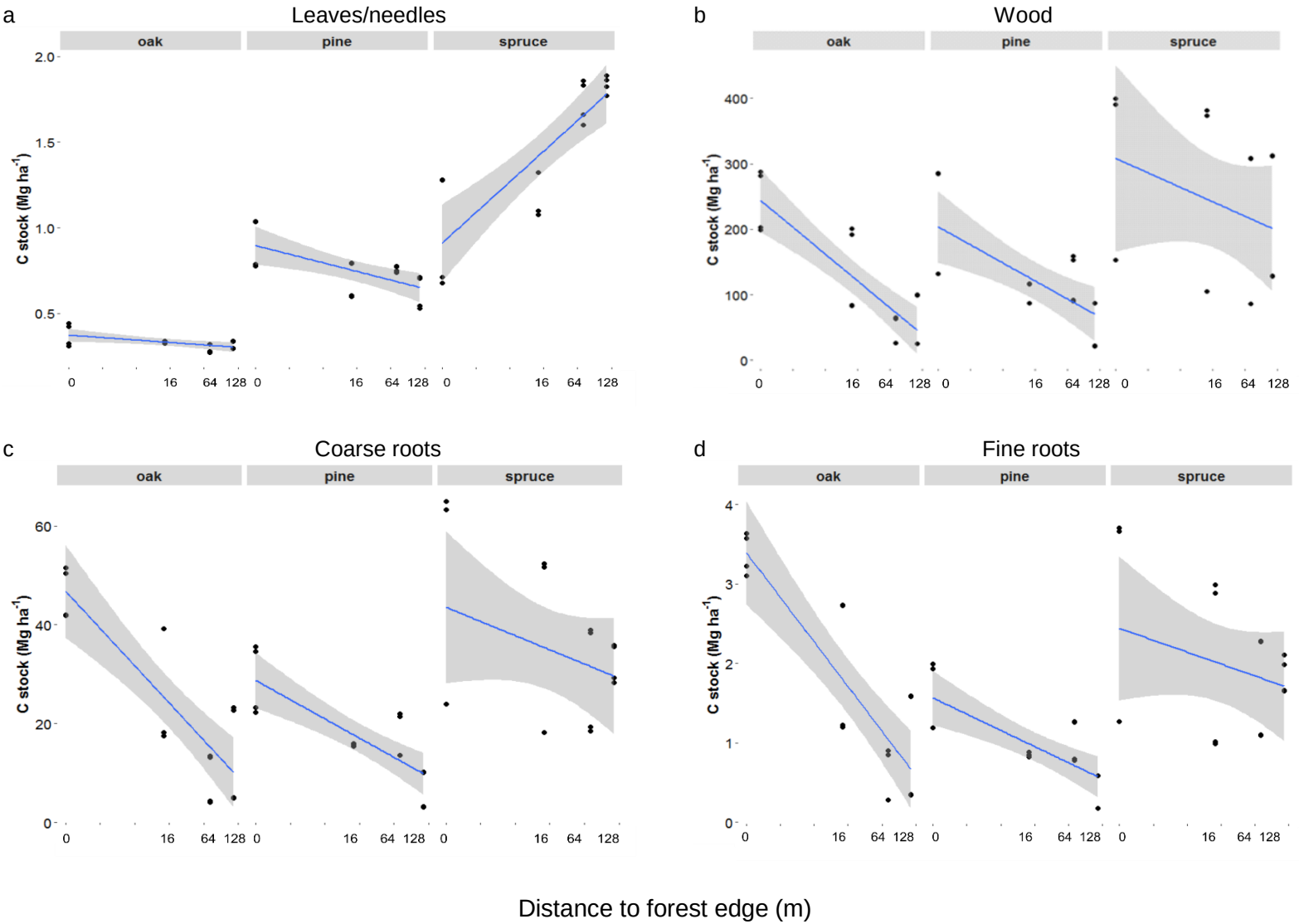

Fig. 2.4: C stock in a) leaves and needles, b) wood, c) coarse roots and d) fine roots (n = 4 at each distance,). Grey shading shows the 95% confidence interval. For readability, x-axes show distance to forest edge in m and not as the logarithm of distance to forest edge. Significant effects of distance to forest edge, forest type and their interaction are presented in Table 2.2.

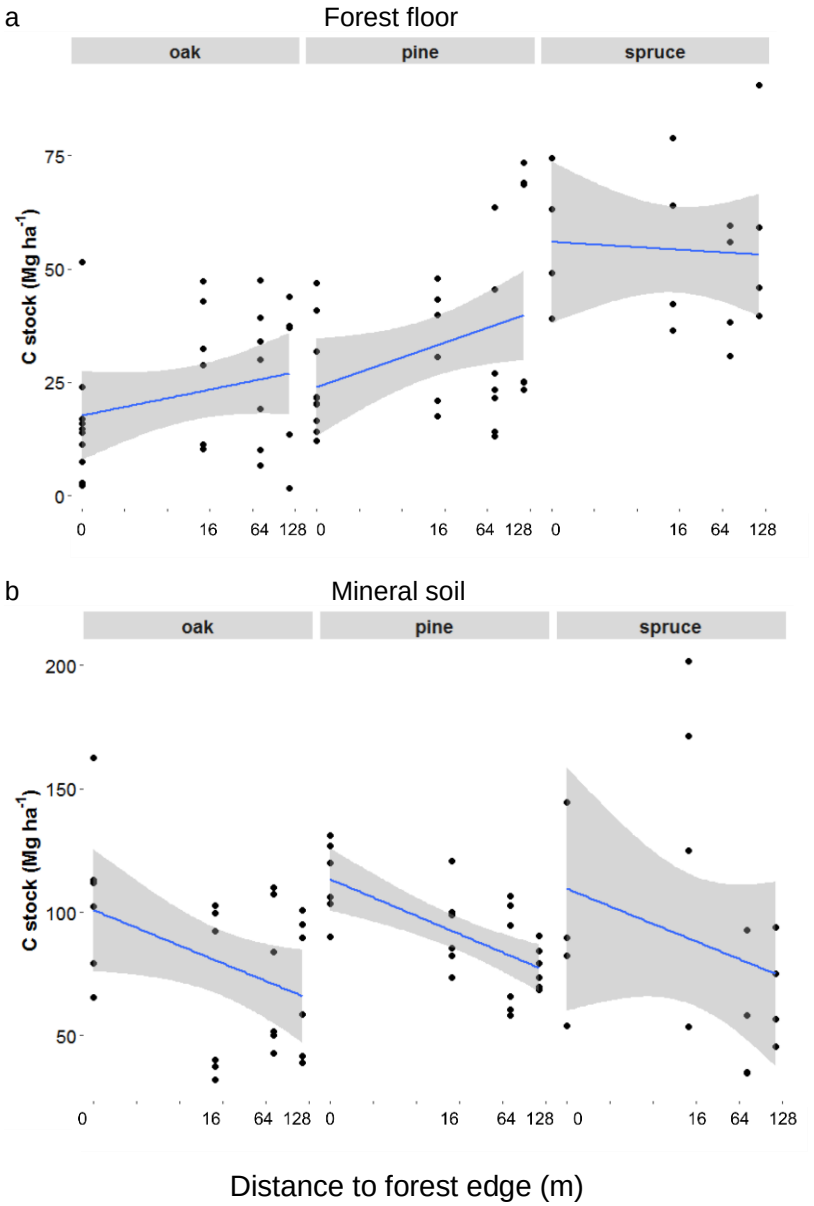

Fig. 2.5: C stock in a) forest floor and b) mineral soil until a depth of 30 cm (n = 4 at each distance, in a) n = 6 in Belgium). Grey shading shows the 95% confidence interval. For readability, x-axes show distance to forest edge in m and not as the logarithm of distance to forest edge. Significant effects of distance to forest edge, forest type and their interaction are presented in Table 2.2.

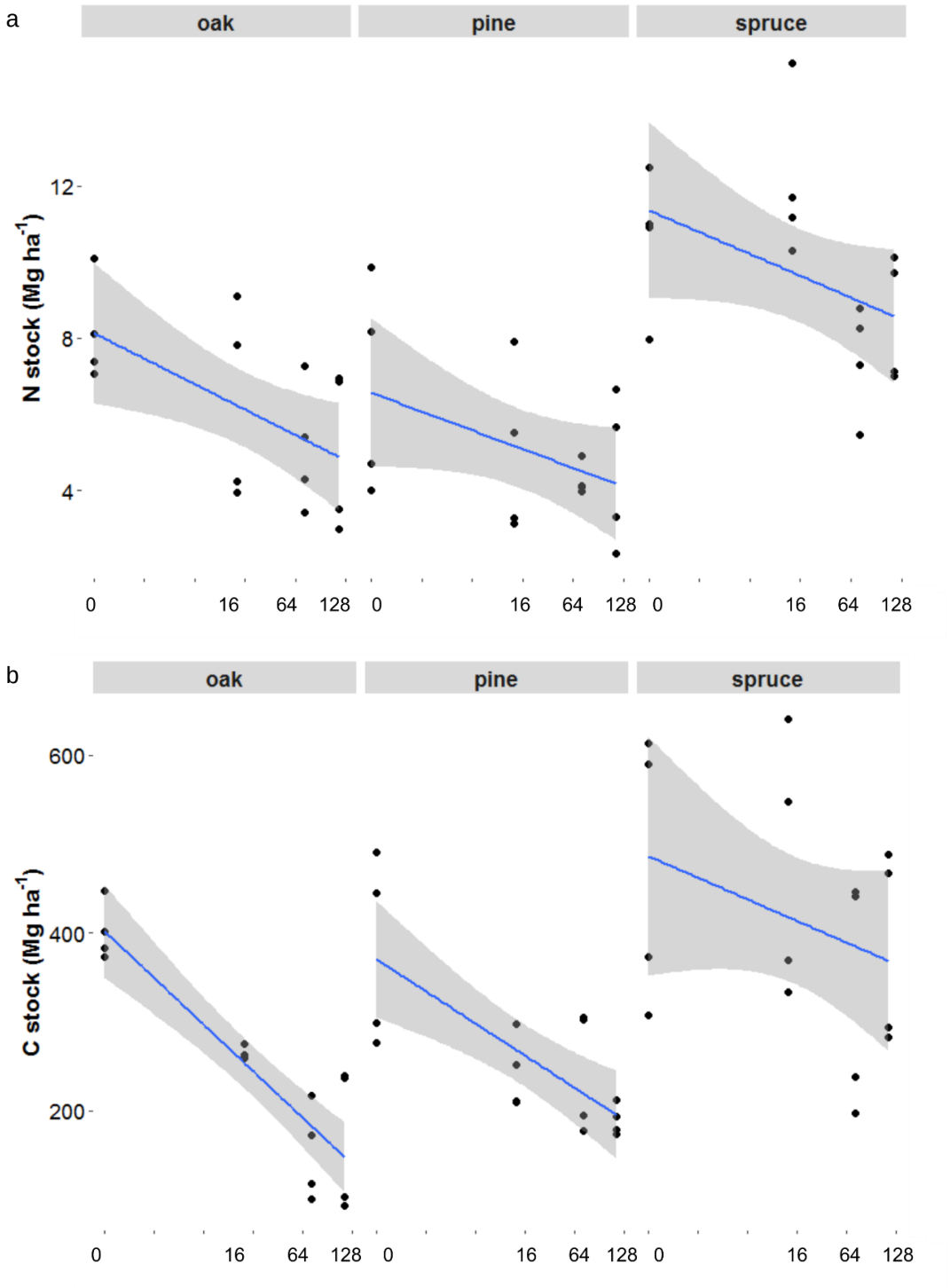

Fig. 2.6: Total stocks for a) nitrogen and b) carbon. Grey shading shows the 95% confidence interval (n = 4 at each distance). For readability, x-axes show distance to forest edge in m and not as the logarithm of distance to forest edge. Significant effects of distance to forest edge, forest type and their interaction are presented in Table 2.2.

2.3.4. Soil carbon sequestration

When calculating soil carbon sequestration (C_{seq}) based on N throughfall deposition, values ranged between -254.6 and 1197.7 kg ha^{-1} yr^{-1} of C (Table 2.3). Soil C_{seq} did not differ significantly between forest edge and interior according to the selected mixed model (Table 2.3). The forest edges (0 m) sequestered on average 646 kg ha^{-1} yr^{-1} of C, while the forest interiors (128 m) sequestered on average 289 kg ha^{-1} yr^{-1} of C. The interaction between distance to the forest edge and forest type significantly affected soil C_{seq} values ($p < 0.05$), while forest type did not ($p > 0.05$). In the pine and spruce stands soil C_{seq} decreased with distance to the forest edge, while the oak stands showed opposite patterns. The oak stand Qr1 was also characterized by a decrease in soil C_{seq} with distance to the forest edge, while in Qr2 soil C_{seq} increased with distance to the forest edge. Soil C_{seq} response to N deposition (C_{resp}) ranged between -8.2 and 27.3 kg of C per kg of N. Positive values indicate that soils work as C sinks, while negative values infer that soils act as C sources.

Table 2.3: a) Net nitrogen immobilization (Δ N), soil carbon sequestration (soil C_{seq}) and C sequestration response to N deposition (C_{resp}) and b) Effects of distance to the forest edge and forest type on soil carbon sequestration.

a	Δ N (kg ha^{-1} yr^{-1})					
Distance	Qr1	Qr2	Pn1	Pn2	Ps	Pa
Edge	18.5	-4.9	40.2	45.5	17.6	40.1
Interior	11.3	3.2	-10.5	26.8	11.6	22.4
	Soil C_{seq} (kg ha^{-1} yr^{-1})					
Edge	316.1	-83.3	897.6	1197.7	496.4	1049.5
Interior	204	55.4	-254.6	697.6	348.1	681.8
	C_{resp} (kg of C kg^{-1} of N)					
Edge	17.1	-2.8	14.8	23.3	18.4	24.4
Interior	11.7	2.4	-8.2	17.6	20.5	27.3

b	R^2	Distance to forest edge	Forest type	Interaction	ICC
Soil C_{seq}[§] (kg ha^{-1} yr^{-1})	0.84	0.065	0.120	**< 0.05** →	0.22

The § indicates a linear mixed effect model was used, with the forest location as random term. R^2 is the coefficient of determination, indicating the proportion of variation explained by the model. The ICC indicates how much of the overall variation in the response is explained by the random term of the mixed model. Bold values are significant ($p < 0.05$). The (→) indicates that the interaction term differs between forest type (meaning that for some forest type values increase with distance to the edge, while they decrease for other forest types).

2.4. Discussion

In this study, we investigated the N and C stocks along an edge-to-interior transect in six temperate forests, comprising of monocultures of oak, pine and spruce. Nitrogen and carbon stocks were increased at the forest edge, confirming our first hypothesis, where we hypothesized that the increased atmospheric N deposition and contrasting microclimate at the forest edge are associated with increased N and C stocks at the forest edges compared to the forest interiors. However, the edge effect was not greater in the selected pine and spruce stands than in the deciduous oak stands. Hence, the second hypothesis, stating that differences in N and C stocks between forest edge and forest interior are more pronounced in the pine and spruce forests compared to the deciduous oak forests can not be confirmed. Ideally, we wanted to disentangle the edge and forest type effect on N and C stocks as they would occur in an unmanaged forest edge. Unfortunately, this was not the case, since the Danish forest edges were subjected to stronger winds than the Belgian forest edges, resulting in a different forest structure at the forest edge. The increased N and C stocks of wood, roots and soil at the forest edge lay in the same range, showing that N and C stocks followed the same patterns. Soil C sequestration values were larger at the forest edge compared to the forest interior (except for Qr2), supporting the third hypothesis, stating that there exists an edge effect on soil C sequestration values in association with N deposition. The close link between N and C is further elaborated in section 2.4.2.

2.4.1. Edge and forest type effect on the N and C stocks of the forest pools

The leaf N and C stocks decreased with distance to the edge in the oak and pine stands, but increased in the spruce stands. This trend can be explained by the LAI, since it decreased as a function of distance to the edge in the oak and pine stands, probably due to more favorable light conditions at the edge, as found by McDonald and Urban (2004), Bowering et al. (2006) and Sherich et al. (2007), but not in the spruce stands. The strong winds in Denmark caused more damage to the spruce trees at the edge than in the interior, thereby lowering the LAI at the edge. The opposite trend in LAI can also be due to the fact that spruce is a shade-tolerant forest type (Seymour and Kenefic, 2002), while pine and oak are more light-demanding forest types. Furthermore, in the *Picea sitchensis* site there was a management road at 130 m from the edge insuring higher light availability for the surrounding trees and thinning debris was deposited along this management road. Needles also had a higher LMA, since the leaf volume per area of needles is larger than leaves (due to larger cell walls and more mesophyll tissue) (Poorter et al., 2009), contributing to the high

N and C stocks (especially in spruce needles) compared to the N and C stocks of the oak leaves.

Nitrogen and C stocks of wood were influenced by the edge effect and its interaction with forest type. Variables that explained the trends in N and C stocks were wood volume and stem density, which were both decreasing with distance to the edge. The forests had a higher wood volume at the edge, indicating that edge characteristics such as increased atmospheric deposition and favorable light conditions positively influenced growth conditions. All forest stands were planted as even-aged monocultures with a constant stem density. The higher stem density at the edge resulted from lower tree mortality rates, but was less pronounced in the spruce stands due to the strong winds and shade-tolerance of spruce trees. Ziter et al. (2014) calculated aboveground C stocks in temperate forest fragments in Quebec, Canada. They obtained constant C stocks, with no effect of edge proximity and attributed this to the interplay of increased tree mortality at the edge due to abiotic stressors and increased tree productivity at the edge due to the contrasting microclimate compared to the forest interior. We can only confirm the latter statement on increased productivity based on our results.

The gradient in the N and C stocks of the coarse and fine roots along the transect can be explained by the gradient in stem density, which contributed in the calculation of the root biomass per surface area and which was highest at the edge and in the spruce stands. The forest type effect was reflected by the root biomass, being highest in the oak stands, due to the higher diameter at breast height used in the allometric relationship of Jenkins et al. (2003). The high ICC values (Table 2.1) showed that variables such as root biomass were dependent on local site characteristics e.g. diameter at breast height. The fine root biomass was calculated based on an estimate of the fine root ratio (0.02 according to Liski et al., 2002), which seems unlikely to apply for all studied forest types. Therefore, the fine root ratio should be used with caution. However, few data exist on fine root biomass. Recently, Jagodzinski et al. (2016) calculated the fine root biomass of 22 *Quercus robur* stands in Poland and obtained a mean fine root biomass of 3.71 Mg ha^{-1}. Their value coincides well with our mean value of the oak fine root biomass (3.59 Mg ha^{-1}), proving the reliability of the fine root ratio for the oak stands in our study. To our knowledge, variations in root biomass along edge-to-interior transects have not been assessed yet.

The forest floor N and C stock were steered by the nutrient concentrations and by the forest floor mass. Nitrogen and carbon concentrations increased with distance to the edge. A possible explanation is that litter degradation was faster due to microclimatic gradients and

a different microbial and invertebrate abundance and community at the edge (De Smedt et al., 2016) and nutrients were transferred to deeper soil layers. Vesterdal et al. (2008) measured C stocks under different temperate forests and found low C stocks in forest floors, where C stocks were high in mineral soil, showing proportional differences in C distribution. The forest floor mass differed between forest type, where the spruce stands had the highest forest floor mass. Their litter is characterized by more recalcitrant components and lower calcium concentrations than broadleaf litter, resulting in lower forest floor turnover (Reich et al., 2005), litter accumulation in the forest floor and formation of acid compounds (Berg, 2000; Hobbie et al., 2007). Furthermore, conifers have shallower rooting systems and tend to accumulate more organic carbon in the forest floor (Jandl et al., 2007).

2.4.2. Edge and forest type effect on soil stocks and C sequestration

Both distance to the edge and forest type influenced soil N and C stocks. The higher N and C stocks at the edge were due to the higher N and C concentrations in the upper 30 cm of the mineral soil (Table 2.1), caused by the possible interplay of higher atmospheric N deposition, microclimatic gradients, higher LAI and changes in microbial and invertebrate abundance and structure (Bowden et al., 2004; Heithecker and Halpern 2007; Hobbie et al., 2007; Sherich et al., 2007). We did not sample deeper soil layers (B horizon, see Fig. A-I), which could affect the soil N and especially soil C stocks, as the B horizon of Podzols is characterized by a high organic matter content (WRB, 2014). Increasing the input of N to a N-limited forest increases initially the photosynthetic capacity of trees (Högberg, 2012) and microbial biomass (Zhang and Zak, 1998). However, over the longer term, these positive effects on growth fade (Ingerslev, 2001) and microbial biomass generally decreases (DeForest et al., 2004). This lack in growth response can be explained by N leaching (Johannisson et al., 1999), storage of excess N in needles, leaves and soil (Persson et al., 2000), but also due to shortages of other essential nutrients such as phosphorus (P) and potassium (K^+) (Eugster and Haeni, 2013). Recent research has shown that stabilization of soil organic matter in recalcitrant forms occurs under N rich conditions (Janssens et al., 2010; Hobbie et al., 2012). The lower soil density at the edges could be explained by a higher organic matter content, as this will inherently also increase soil pore space (Arvidsson, 1998). Ginzburg (2014) also measured the effects of N deposition on C stocks at a finer spatial resolution in the two Danish spruce stands Ps and Pa. These forests were not characterized by nitrate leaching and had higher C stocks near the edges (although not significant), indicating a positive effect on C stocks when forest are exposed to moderate rates of N deposition (Thomas et al., 2010). We found a significant edge effect in the six

investigated forest edges. Strong microclimatic gradients exist in recently exposed forest edges, according to Matlack (1993), but these effects recede over time. The impact of edge orientation was controlled for by selecting forest edges which are all facing west, the dominant wind direction. Along the transects in our well-developed forest edges small, but significant differences in soil temperature and moisture existed (unpublished data). Forest edges (0 - 2 m) tended to be warmer and drier than forest interiors (64 - 128 m) (except for the *Picea* sites, which were wetter at the edge due to lower interception of rain) affecting decomposition rates of soil organic matter (Conant et al., 2011) and contributing to the differences in N and C stocks between forest edges and interiors. Decomposition rates in spruce forests are low compared to hardwood species (Berg 2000), explaining the higher N and C stocks in the spruce forests.

Magnani et al. (2007) found a very strong correlation between N deposition and net C sequestration of forest ecosystems, where several hundreds of kg of C were sequestered per kg of N deposited on the forest. The potential C fixation response to N deposition is restricted by the C/N stoichiometry of the forest ecosystem pools (De Vries et al., 2009). The results of Magnani et al. (2007) imply that deposited N would solely lead to an increase in stem wood, which is the only C sink with a C/N ratio around 500 (de Vries et al., 2008). However, ^{15}N-labelled tracer experiments in temperate forests indicated that most N retention occurs in soil (Nadelhoffer et al., 1999). Furthermore, Magnani et al. (2007) overlooked the internal N supply and attributed the high C retention only to wet N deposition (Högberg, 2012) and did not take N losses, such as N leaching and N trace gas emission, into account (De Schrijver et al., 2008). De Vries et al. (2008) obtained a soil response of 10 to 30 kg C per kg N under a total N deposition of 10 to 25 kg ha^{-1} yr^{-1} of N. Since the C and N cycle are closely linked, the N accumulation can be used to approximate C sequestration, i.e. the 'N balance method' (Gundersen et al., 2006). In our calculation, N trace gas emission (which is expected to be low, as was the case in Qr2 and Pn2, see Chapter 3) and organic N leaching were neglected. According to Sleutel et al. (2009) the leaching losses of dissolved organic N can be substantial (9-28% of the total amount of N leached). Vanguelova et al. (2010) also measured high DON leaching (30 % of the total amount of N leached) in 10 Level II monitoring plots in the UK. It should be noted that yearly inorganic N leaching of the spruce stands Pa and Ps was obtained via an empirical relationship (Eq. 2.13), which might deviate from the actual leaching losses. This relationship was based on intensive monitoring of 8 forest plots during 3 to 4 years. The observed correlation showed to be very strong (R^2 = 0.88). Equation 2.13 consists of an intercept, which statistically gave the best fit. However, it would have been better to force the correlation through zero. The C

sequestration response to N deposition lay in the range reported by de Vries et al. (2008), except for two low values in Qr2 and Pn1 (Table 2.3). The edge of Qr2 and interior plots of Pn2 were characterized by high N leaching values, which could be linked to the vicinity of a ditch, facilitating losses of dissolved N. Our soil C sequestration values were higher at the edge, explaining the higher C stocks. However, it is unclear what the fate of this accumulated C is on the long-term.

When looking at a broader scale, i.e. Flanders (northern Belgium), De Schrijver et al. (2007) showed the importance of incorporating forest edges in the evaluation of surpassing critical pollutant loads. They considered 58 % of the total forested area in Flanders as external forest edges, bordering a non-forested area, based on forest inventory data of the Bosreferentie and a median forest edge distance of 50 m (Aminal afdeling Bos en Groen 2001). De Schrijver et al. (2007) calculated that surpassing critical load values of N deposition was underestimated by 31 % when forest edge effects were not included. When looking at the total mean N and C stock in the forest interior, excluding the influence of forest edge, we obtained 5.8 Mg ha^{-1} of N and 251 Mg ha^{-1} of C. However, when taking the forest edge effect into account, using the same forest edge area as De Schrijver et al. (2007), we obtained a mean N and C stock of 7.5 Mg ha^{-1} of N and 365 Mg ha^{-1} of C showing an underestimation of respectively, 22 % and 31 % when N and C stocks are calculated on regional or national scales based on data from forest interiors only. Our findings underline the need to include forest edges in programs monitoring forest C changes, since huge amounts of C can potentially be stored in these edges. Additional research in temperate forest edges is needed to provide an adequate knowledge of their N and C storage capacity and long-term behavior.

2.5. Conclusion

The forest edges of our study sites in Belgium and Denmark stored more N and C than forest interior zones. Based on our results, the edge effect on N and C stocks was not more pronounced in forest edges of the coniferous pine and spruce stands than of the deciduous oak stands. Our results confirmed the strong link between the N and C cycle, showing the association between N deposition and soil C sequestration. Up till now, the increased N and C stocks at forest edges were stored in all forest pools (except the forest floor) and mainly in the mineral topsoil and woody biomass, but it is unclear for how long this will last. Forest edges are a dominant feature in many landscapes of Central and Western Europe. Hence more research should be conducted to gain better insight in nutrient cycles at forest edges. The capacity of higher C retention at the edge can influence the rates and balances of C storage and, hence, correct C sequestration assessments.


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3. Edge effect on fluxes of N and C trace gases

After: Remy E., Gasche R., Kiese R., Wuyts K., Verheyen K., Boeckx P. (2016). Edge effects on N_2O, NO and CH_4 fluxes in two temperate forests. Science of the total environment 575: 1150-1155.

Abstract

Forest ecosystems may act as sinks or sources of nitrogen (N) and carbon (C) compounds, such as the climate relevant trace gases nitrous oxide (N_2O), nitric oxide (NO) and methane (CH_4). Forest edges, which catch more atmospheric deposition, have become important features in European landscapes and elsewhere. Here, we implemented a fully automated measuring system, comprising static and dynamic measuring chambers determining N_2O, NO and CH_4 fluxes along an edge-to-interior transect in an oak (*Q. robur*) and a pine (*P. nigra*) stand in northern Belgium. Each stand was monitored during a 2-week measurement campaign with continuous measurements every 2 hours. NO emissions were 9-fold higher than N_2O emissions. The fluxes of NO and CH_4 differed between forest edge and interior, but not for N_2O. This edge effect was more pronounced in the oak than in the pine stand. In the oak stand, edges emitted less NO (on average 60 %) and took up more CH_4 (on average 177 %). This suggests that landscape structure can play a role in the atmospheric budgets of these climate relevant trace gases. Soil moisture variation between forest edge and interior was a key variable explaining the magnitude of NO and CH_4 fluxes in our measurement campaign. To better understand the environmental impact of N and C trace gas fluxes from forest edges, additional and long-term measurements in other forest edges are required.

3.1. Introduction

Atmospheric trace gases, such as carbon dioxide (CO_2), methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O) and nitric oxide (NO), are defined as minor constituents of the atmosphere, occurring at smaller mixing ratios than nitrogen (N_2) and oxygen (O_2) (Conrad, 1994). Nitrous oxide, NO and CH_4 are important greenhouse gases (GHGs), with N_2O and CH_4 being direct GHGs and NO contributing indirectly to climate change. Deposition of reactive nitrogen (N) species has increased worldwide owing to anthropogenic activities, such as the use of fossil fuels and agricultural production (Aardenne et al., 2001). Ammonia (NH_3) and N oxides (NO_x) are emitted from agricultural systems, traffic and industry and may be transported off-site and fertilize other systems which can lead to enhanced production of N_2O (Pilegaard et al., 2006). Oxic soils (e.g. forests, grassland) are regarded as the only biological sink of atmospheric CH_4 (Dutaur and Verchot, 2007). From these soils, forests consume the most atmospheric CH_4 (Dutaur and Verchot, 2007) and are now recognised as a major contributor to CH_4 oxidation in terrestrial ecosystems (Wang and Ineson, 2003).

Forest edges have become important features in European landscapes (Hofmeister et al., 2013) and differ substantially from forest interior zones in terms of microclimate via air and soil temperature, light availability, soil moisture and wind speed, and atmospheric deposition (e.g. Marchand and Houle 2006; Wuyts et al. 2008a, 2008b, see § 1.1 and 1.4). Most studies on N and C trace gases so far have been conducted in the forest interior. However, edge effects generating gradients in N deposition and microclimate may bias regional estimates, especially in highly fragmented landscapes.

Regarding microclimate, soil moisture is a key variable affecting the emission rates of N and C trace gases (Firestone and Davidson, 1989). Soil water acts as a transport medium for nitrate (NO_3^-) and ammonium (NH_4^+) and influences the rate of O_2 supply, thereby controlling whether aerobic processes such as nitrification and methane uptake or anaerobic processes such as denitrification and methane production dominate within the soil profile. At a water filled pore space (WFPS, the ratio of volumetric soil water content to total soil porosity) below 50 %, an increase in soil moisture will increase NO emission, while at a WFPS higher than 60 %, NO emission decreases due to the reduced diffusion efficiency of the gas and increasing dominance of denitrification (Davidson et al., 2000; Fig. 3.1). Other soil physicochemical factors such as pH, temperature, mineral N and organic matter content are also considered to influence trace gas fluxes (Wang and Ineson, 2003; Rütting et al., 2013).

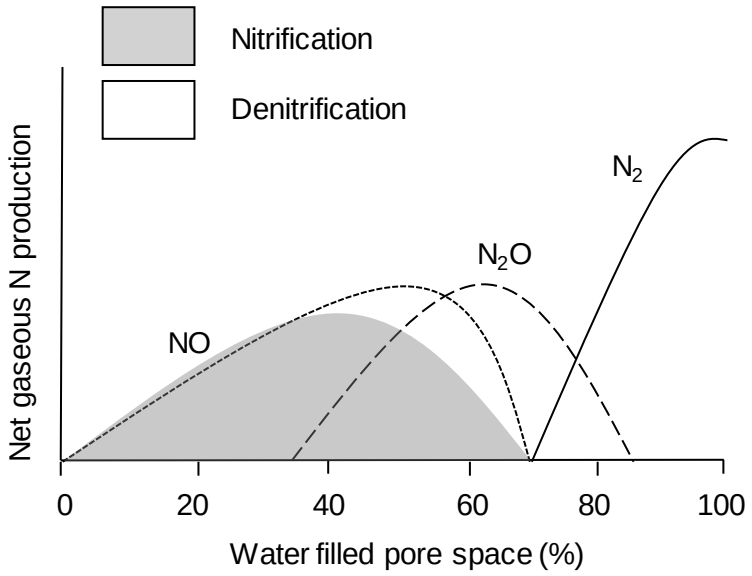

Fig. 3.1: Conceptual figure of the relative contribution of nitrification (grey area) and denitrification (white area) to NO, N_2O and N_2 emissions in function of soil water filled pore space (WFPS, %) (adapted from Davidson et al., 2000).

Many studies have investigated the effects of elevated atmospheric N deposition on forest biogeochemistry. Butterbach-Bahl et al. (2002) measured an increase in NO and N_2O fluxes at higher N-affected sites (22 kg N ha^{-1} yr^{-1}) as compared to sites with moderate atmospheric N input (15 kg N ha^{-1} yr^{-1}). Skiba and Smith (2000) identified the interacting effects of substrate supply (as N additions or mineralisation of organic N), soil moisture and temperature as the key drivers of N_2O emission. In addition, Butterbach-Bahl et al. (2002) demonstrated that in pine forest sites with moderate atmospheric N deposition CH_4-uptake rates were two- to five-fold higher than at the sites with high atmospheric N input. It has generally been accepted that the consumption of CH_4 in soils is inhibited by nitrogenous fertiliser additions (Smith et al, 2000; Jassal et al., 2011). Besides the oxidation of CH_4, the enzyme methane monooxygenase also has the ability to convert ammonia to nitrite (Bodelier and Laanbroek, 2004). However, depriving methane-oxidising bacteria of a N-source hampers their growth and activity. Hence, in N-limited conditions the oxidation of CH_4 can be stimulated by the addition of N (Papen et al., 2001; Bodelier and Laanbroek, 2004).

In this study, we investigated the fluxes of N_2O, NO and CH_4 along an edge-to-interior-transect in an oak (*Q. robur*) and a pine (*P. nigra*) stand, which are common tree species in the studied region. We hypothesized that due to the enhanced atmospheric N deposition, contrasting microclimate (soil moisture and temperature) and soil physicochemical variables (pH, C/N) at the forest edge versus interior, nitrogen oxide emissions are increased and CH_4 uptake is decreased while moving from the forest edge to the interior.

3.2. Material and methods

3.2.1. Study site and experimental set-up

The study was performed in two (Qr2 and Pn2) of the six selected forest edges, described in chapter 1 (§ 1.6). An overview of the stand and physicochemical characteristics of the oak stand Qr2 and the pine stand Pn2 can be found in Table 1.1. Briefly, mean annual air temperature is 10.5°C and mean annual precipitation is 800 mm (data obtained from a nearby weather station operated by the Royal Meteorological Institute of Belgium, 1981 - 2010). Both forest stands are even-aged monocultures and grow on acid, quartz-dominated Podzols (Wuyts et al., 2008b). Previous land use was heathland until afforestation in last century (1939 for Qr and 1964 for Pn2). The considered forest edges are facing the locally prevailing wind direction (west to southwest), which creates the steepest throughfall deposition gradients (Draaijers et al., 1988). Leaf area index (LAI) (± standard deviation) in the oak stand is 2.1 ± 0.5 and 1.9 ± 0.1, respectively in the edge and interior, and is 2.8 ± 0.2 and 1.8 ± 0.1, respectively in the edge and interior of the pine stand (Wuyts et al., 2011).

In each stand, four blocks were delineated: two blocks (block 1 and 2) were situated in the edge (0 – 5 m) and two blocks (block 3 and 4) were in the forest interior (64 m, Fig. 3.2). In total, twenty chambers for measuring gas fluxes were divided over the four blocks, so that each block consisted of five chambers: three static chambers for N_2O/CH_4 flux measurement and two dynamic chambers for NO_x flux measurement. The minimum distance between the chambers within each block was 3 m. Understory vegetation was naturally absent at the chamber positions.

Reference chambers were used for determination of ambient air concentrations of N and C trace gases. The steering boxes, controlling opening, closing and air sampling of the chamber headspace, were placed in a central position between the edge and interior blocks (at 32 m from the edge). Due to the novel and explorative character of this study, each stand was monitored continuously for only two weeks (from 23 April until 8 May 2014 in the oak stand and from 9 to 23 May 2014 in the pine stand). Additional soil moisture (TDR probe CS 625-L, Campbell Scientific, United Kingdom) and temperature (iButton DS1921G, Fondriest Environmental, USA) measurements were executed every 2 hours at a depth of 5 cm on an edge-to-interior transect parallel to the measuring chambers. By using these measuring chambers we focused on soil fluxes of N and C trace gases, as it was not our aim to obtain an inventory of the GHG emissions of the whole ecosystem.

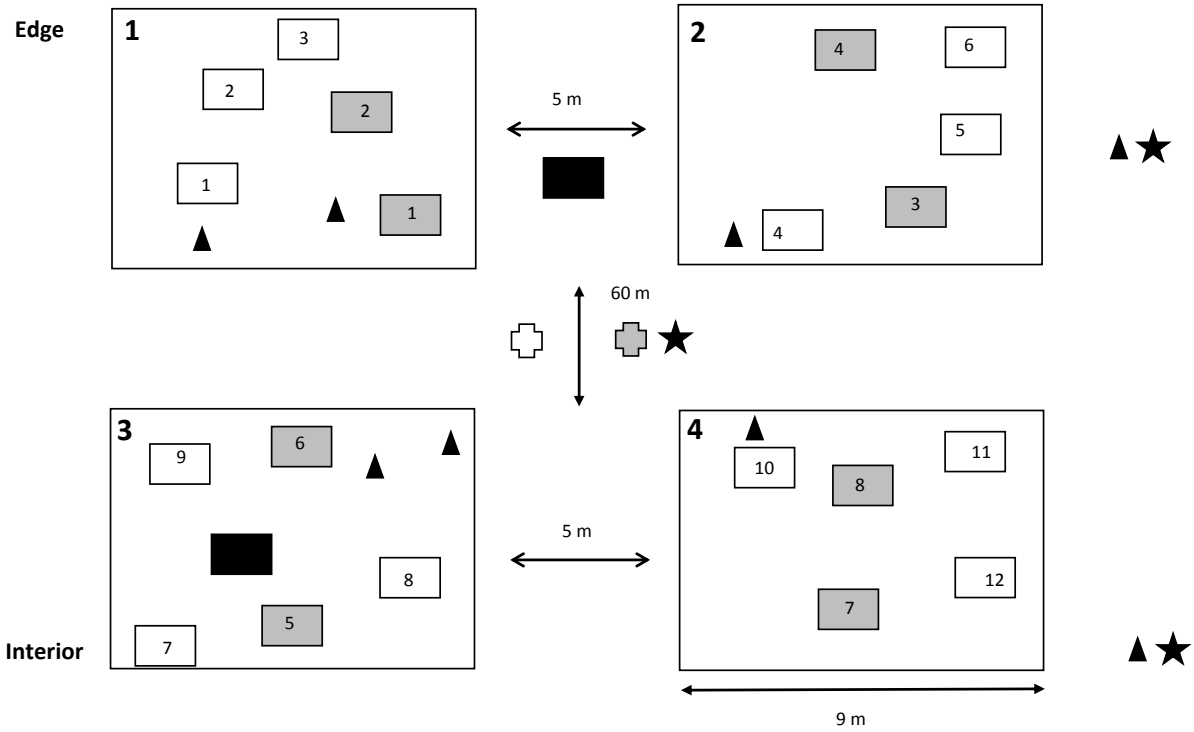

Fig. 3.2: Experimental set-up used to measure N_2O, NO and CH_4 fluxes. Black = reference chamber, white = N_2O/CH_4 static chamber, grey = NO dynamic chamber, white cross = N_2O/CH_4 steering box, grey cross = NO steering box. Numbers 1 to 4 refer to the respective blocks (block 1 and 2 = edge, block 3 and 4 = interior). In total 12 static chambers (numbers 1 to 12) and 8 dynamic chambers (numbers 1 to 8) were used in each forest. ▲ = soil moisture measurement, ★ = soil temperature measurement.

3.2.2. Measurement of N_2O, NO and CH_4 fluxes

For determination of N_2O, CH_4 and NO_X fluxes a fully automated measuring system was used, described in detail by e.g. Papen and Butterbach-Bahl (1999), Rosenkranz et al. (2006) and Wu et al. (2010). Gas samples for N_2O and CH_4 were taken at a rate of 200 ml min^{-1} and water vapour and CO_2 were removed prior to analysis. Air samples were analyzed by a Shimadzu GC 17A gas chromatograph (GC) equipped with an electron capture detector (ECD) for detection of N_2O and flame ionization detector (FID) for detection of CH_4. For NO_X flux measurements, ambient air was sucked at a constant rate (54 l min^{-1}) across the surface of the chambers by a sampling pumps and transported via PTFE tubings to a NO_x analyser consisting of a chemoluminescence detector CLD 88p and a photolysis converter PLC 860 (both Ecophysics AG, Switzerland). All PTFE sampling tubings were surrounded by black, light impermeable PE tubing, in order to exclude photolysis of NO_2 within the tubings. The IFU data acquisition system for Windows (IDASw) was used to govern and control the automatic measuring system and to acquire and store all data. Both

detectors of the gas chromatograph were automatically calibrated every 18 minutes analyzing standard gas (0.4 ppm N_2O and 4.0 ppm CH_4 in synthetic air, Air Liquide, Germany). Calibration of the NO_X analyzer was performed weekly using 40 ppb NO in synthetic air produced by dilution of recalibrated standard gas (4 ppm NO in N_2, Air Liquide, Germany) with synthetic air (Air Liquide, Germany) using a computerized multi-gas calibration system (Environics 6100, Environics Inc., USA).

The static chambers within blocks 1 and 2 (edge) or 3 and 4 (interior) were closed for 96 minutes each during which 4 air samples were taken and analysed for N_2O and CH_4 concentration changes over time and used for linear flux calculation. Every dynamic NO_X chamber was closed and measured for 6 minutes and thereafter opened, and before each sampling of a measuring chamber, the reference chamber was sampled. Within each 6 minutes sampling time, concentrations of NO, NO_2 and O_3 were determined two times. Corrections for initial concentrations of NO, NO_2 and O_3 at the outlet of each chambers and calculation of fluxes of NO and NO_2 were performed according to Butterbach-Bahl et al. (1997). Ozone concentrations were determined simultaneously to NO and NO_2 using an infrared ozone analyzer (TE49C, Thermo Environmental Instruments Inc., USA). All instruments and computers used were located inside a measuring truck and electrical power was provided by a power generator (12 kW) placed 150 meters away (downwind) from the actual measuring sites.

3.2.3. Soil analysis

At the end of the trace gas flux measuring campaigns, ectorganic layer and mineral soil samples were taken at each chamber position. Mineral soil samples were taken with a soil auger to a depth of 30 cm. A subsample of 5 g sieved fresh soil was extracted with 10 ml 1 M KCl, shaken for 1 h (150 rpm), and filtered (Schleicher & Schuell Microscience 598 ½) prior to analysis of NH_4^+ and NO_3^- concentrations. Ammonium was determined colorimetrically by the salycilate-nitroprusside method (Mulvaney, 1996) on an auto-analyzer (AA3, Bran & Luebbe, Germany). Nitrate was determined colorimetrically using the same auto-analyzer after reduction of NO_3^- to nitrite (NO_2^-) in a Cu-Cd column, followed by the reaction of NO_2^- with N-1-napthylethylenediamine to produce a chromophore. The remainder of the mineral soil samples at each chamber position were pooled, dried for 48 h at 40 °C and analysed for N and C concentration with a CNS elemental analyzer (Vario Macro Cube, Elementar, Germany). Ectorganic layer (including litter, fragmented and humified litter) samples were dried for 48 h at 65 °C and ground (ZM1, Retsch, Germany),

before being analysed by the same CNS elemental analyzer. All samples were analysed for pH-H_2O (pH meter Orion 920A with pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA) by using a 1:5 ratio and shaking the diluted samples for 5 min at 300 rpm. At each chamber position, thickness of the ectorganic layer was determined with a folding rule. The WFPS was calculated as described in Haney and Haney (2010).

3.2.4. Statistical analysis

Spatial variability of the gas fluxes within one forest was determined by calculating the coefficient of variation (c_v) via Eq. (3.1)

$$Coefficient\ of\ variation\ (c_v)(\%) = \sigma/\overline{x} * 100 \quad \text{(Eq. 3.1)}$$

where σ is the standard deviation and $\overline{x}$ is the mean flux (both expressed as µg m^{-2} h^{-1}) calculated from the data of all chambers within one stand. The oak and pine stand were analysed separately. All statistical analyses were performed with R using package lme4. A linear mixed-effect model was used to assess the effect of the discrete predictor variable, edge proximity (edge/interior), on trace gas fluxes. The model comprised a random term where measuring chamber was nested within block, representing the hierarchy of the set-up. The need for a mixed-effect model was verified by comparison with a linear model, comprising edge proximity as predictor variable. The appropriate model was chosen based on the Akaike Information Criterion (AIC) values. The influence of the soil physicochemical variables (soil moisture, soil temperature, N throughfall, C/N ratio and pH of litter layer and mineral soil, NH_4^+ and NO_3^- concentrations of mineral soil and litter depth) on N and C trace gas fluxes was also tested via mixed-effect models. The relationship between the fitted values and the residuals of each model was checked to ensure normality and homoscedasticity.

3.3. Results

3.3.1. Soil physicochemical variables

In both stands, the edge was significantly drier ($p < 0.05$) than the interior throughout the measurement campaign. Mean water filled pore space (WFPS) was 18 and 27 % respectively, at the edge and interior of the oak stand and 11 and 29 %, respectively, at the edge and interior of the pine stand. During the experiment, soil temperature differed significantly between edge and interior of both stands (12.5 and 10.5 °C, respectively at the edge and interior of the oak stand, $p < 0.001$ and 14.0 and 13.0 °C at the edge and interior

of the pine stand, $p < 0.001$). Total rainfall during the experiment amounted up to 88 mm and 189 mm, in the oak and pine stand, respectively. Correlation analyses (data not shown) revealed that soil moisture positively influenced NO emission in both stands ($r = 0.76$, $p < 0.05$, $n = 16$), while it negatively influenced CH_4 uptake ($r = 0.31$, $p < 0.01$, $n = 24$). Nitrous oxide emissions were unaffected by soil moisture ($p > 0.05$). Soil temperature did not affect N and C trace gas fluxes ($p > 0.05$). Carbon to N ratios, soil pH values, soil NH_4^+ and NO_3^- concentrations and litter depth and their influence on the measured N and C trace gas fluxes are shown in Table 3.1.

Table 3.1: a) Mean physicochemical variables (± standard deviation) of the ectorganic layer (el) and the mineral soil (ms) in the oak and the pine stand and b) significance of the effects of these physicochemical variables on the N and C trace gas fluxes according to the linear mixed-effects model outcome. Bold values are significant ($p < 0.05$), (+) positive correlation, (-) negative correlation.

a	Location	C/N ratio el	C/N ratio ms	NH_4^+ concentration (mg kg^{-1}) ms	NO_3^- concentration (mg kg^{-1}) ms	pH (H_2O) el	pH (H_2O) ms	Ectorganic layer (cm)
Oak	Edge	16.4 ± 0.5	13.8 ± 0.8	10.4 ± 0.9	5.9 ± 3.2	4.3 ± 0.2	4.2 ± 0.1	4.4 ± 1.5
	Interior	17.0 ± 0.8	14.4 ± 1.0	9.7 ± 0.8	9.5 ± 3.5	4.1 ± 0.2	4.0 ± 0.1	7.2 ± 1.1
Pine	Edge	25.3 ± 2.1	18.5 ± 1.5	9.4 ± 0.8	1.0 ± 0.4	4.0 ± 0.2	3.9 ± 0.1	5.6 ± 2.6
	Interior	24.9 ± 2.4	19.5 ± 1.7	8.8 ± 1.2	0.6 ± 0.2	4.0 ± 0.1	4.1 ± 0.2	5.8 ± 1.6
b								
N_2O emission		0.734	**< 0.001 (-)**	**< 0.001 (+)**	**< 0.01 (+)**	0.972	0.391	0.136
NO emission		0.274	0.057	0.176	0.056	0.827	0.285	0.191
CH_4 uptake		0.538	**< 0.01 (+)**	0.148	0.877	**< 0.01 (-)**	0.052	**< 0.05 (+)**

3.3.2. Nitrous oxide emission

There was no significant effect of edge proximity in the oak stand ($p > 0.05$, Fig. 3.3a), i.e. N_2O emissions of forest edges (mean ± standard deviation 6.8 ± 2.4 µg N m^{-2} h^{-1}) did not differ from forest interiors (7.7 ± 2.3 µg N m^{-2} h^{-1}). The spatial variability of N_2O emission between measuring chambers was high, with a c_v of 50 % over all blocks. A significant effect of edge proximity was also lacking in the pine stand ($p > 0.05$), with a mean N_2O emission of 2.6 ± 1.4 µg N m^{-2} h^{-1} at the edge and 1.7 ± 1.5 µg N m^{-2} h^{-1} at the interior (Fig. 3.3b). The spatial variability of N_2O emission between measuring chambers in the pine stand was very pronounced, with a c_v of 124 % over all blocks.

3.3.3. Nitric oxide emission

Nitric oxide emissions differed significantly between the edge and interior of the oak stand ($p < 0.01$, $R^2 = 0.55$, Fig. 3.3c), with higher NO emission in the forest interior. The mean NO emission (± standard deviation) was 43.5 ± 11.4 µg N m^{-2} h^{-1} at the edge and 105.9 ± 38.6 µg N m^{-2} h^{-1} at the interior, being on average 60 % lower at the forest edge. The c_v over all blocks is of the same magnitude as the spatial variability in N_2O emission, i.e. 49 %. There was no effect of edge proximity in the pine stand, with a mean NO emission of 35.5 ± 21.6 µg m^{-2} h^{-1} at the edge and 11.7 ± 14.9 µg m^{-2} h^{-1} at the interior ($p > 0.05$, Fig. 3.3d). The spatial variability of the NO emission between measuring chambers in the pine stand was high, with a c_v of 79 % over all blocks. In both stands, the NO emissions were approximately 9 times higher than N_2O emissions.

3.3.4. Methane uptake

In the oak stand, edges took up more CH_4 than interior sites ($p < 0.05$, $R^2 = 0.60$, Fig. 3.3e). The mean CH_4 uptake (± standard deviation) was -59.6 ± 19.2 µg m^{-2} h^{-1} at the edge and -21.4 ± 10.8 µg m^{-2} h^{-1} at the forest interior, being on average 177 % higher at the forest edge. There was no significant effect of edge proximity in the pine stand, since mean CH_4 uptake was -22.7 ± 7.8 µg m^{-2} h^{-1} at the edge and -16.6 ± 5.3 µg m^{-2} h^{-1} at the forest interior ($p > 0.05$, Fig. 3.3f). Among the trace gases studied, the spatial variability of CH_4 uptake rates was the least pronounced (indicated by the low c_v, i.e. 31 % in the oak stand and 38 % in the pine stand).

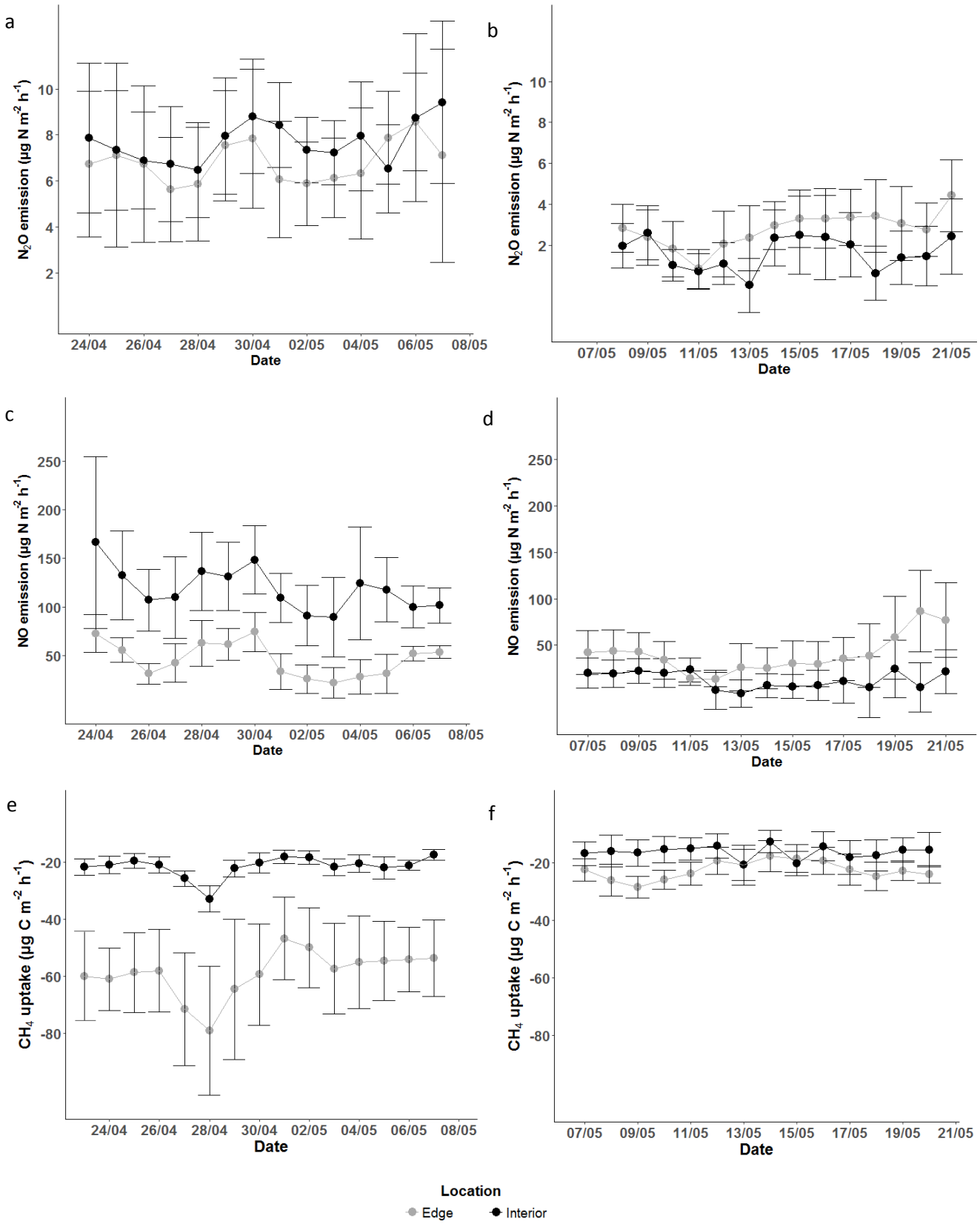

Fig. 3.3: Mean daily N_2O emission, NO emission and CH_4 uptake at the edge and interior of the oak (a,c,e) and pine stand (b,d,f). Error bars indicate standard deviations.

3.4. Discussion

In this study, we investigated N and C trace gas fluxes along an edge-to-interior transect in two temperate forest stands, comprising monocultures of oak and pine. Most studies on N and C trace gases measure several year-round fluxes to observe intra- and inter-annual fluctuations. However, we preferred to perform an explorative measurement campaign at high temporal resolution to get a first insight in the - up to now - unexplored edge effect on N and C trace gas fluxes, and therefore chose to measure continuously (every two hours) during two weeks in each stand. As the number of measurement chambers was limited, the experimental set-up (Fig. 3.2) could not be used in the oak and pine stand within the same monitoring period. Therefore, we did not aim at comparing N and C trace gas fluxes from the oak and the pine stand. High coefficients of variation (c_v) of the N and C trace gases, inherent to trace gas fluxes, were observed. Nevertheless, clear signals were detected.

3.4.1. Edge effects on NO and N_2O emission

In the oak stand, there was an edge effect on NO emission, with lower NO emissions at the forest edge. Although edges in the pine stand emitted on average more NO than the interior, the edge effect on NO emission was not significant in the pine stand due to the large variability. In the forest interior, LAI was lower and understory vegetation was less dense, causing less rainfall interception, leading to higher throughfall volumes in the interior in the oak stand (Wuyts et al., 2011). Therefore, higher soil moisture values in the forest interior probably led to more optimal conditions for NO emissions. In this study, NO emissions were approximately 9 times higher than N_2O emissions. The oak stand was monitored from the end of April until the beginning of May, which coincides with the period of oak leaf development. Therefore, the maximum LAI was probably only reached at the end of the monitoring period, which could have influenced our results.

There was no effect of edge proximity on N_2O emission rates in the oak stand, nor in the pine stand, although we hypothesized that enhanced atmospheric N deposition and contrasting microclimate at the forest edge versus interior would increase N_2O emissions. In our studied stands, N_2O emissions were rather low. The WFPS values were probably too low to obtain substantial N_2O emissions (Fig. 3.1). Nitrous oxide emissions might increase in wetter and warmer conditions, as a rise in soil temperature increases the rates of enzymatic processes as long as other factors, such as substrate availability or moisture have no limiting effect (Szukics et al., 2010). Higher availability of inorganic N (NH_4^+ and NO_3^-) stimulated N trace gas emission, as this is the substrate of nitrification and denitrification

(Firestone and Davidson, 1989). Several authors did find significant differences in substrate availability between forest edge and interior. Spangenberg and Kölling (2004) measured lower C/N ratios of the forest floor due to enhanced N deposition in the edge of a spruce forest, exposed to high NH_3 emission in Germany. Wuyts et al. (2008b) also measured higher N deposition at the edges of the oak and pine stand used in this research, compared to the forest interior.

3.4.2. Edge effects on CH_4 uptake

Methane uptake was higher in the edge of the oak stand than in the forest interior, while there was no significant edge effect in the pine stand. This contradicts our hypothesis, that due to the enhanced atmospheric N deposition and contrasting microclimate at the forest edge, CH_4 uptake would be lower in the forest edge than in the forest interior. Many reports have shown that CH_4 uptake is inhibited by N that is added to soils (King and Schnell, 1998; Bodelier and Laanbroek, 2004). Instead, we found higher CH_4 uptake in the edges, where N deposition is increased. This has also been observed by Bodelier et al. (2000) and De Visscher and Van Cleemput (2003), who attributed this increase to the type of methanotrophic bacteria, who became N limited after the depletion of inorganic N due to their cell synthesis. Furthermore, soil moisture negatively influenced CH_4 uptake, as beyond a soil moisture optimum more soil moisture will limit O_2 and CH_4 diffusion (Wang and Ineson, 2003), favouring CH_4 uptake at the drier oak forest edge. Hütsch (2001) found that methanotrophic activity decreased with low soil pH, while this was linked to the acidifying effect of NH_4^+ deposition. A dense litter layer may also limit CH_4 uptake as gas transport is determined by diffusion (Adamsen and King, 1993). Methane emission from litter induced by UV radiation (Bruhn et al., 2012; Vigano et al., 2008) might have slightly impacted the CH_4 flux.

3.5. Conclusion

The fluxes of NO and CH_4 differed between forest edge and interior, and this edge effect was more pronounced in the oak than in the pine stand. Our results at the oak stand indicated that forest edges emitted less NO and took up more CH_4 (on average respectively 60 % and 177 %). Consequently, landscape structure can play a role in the atmospheric budgets of these climate relevant trace gases. Soil moisture variation between forest edge and interior was a key variable explaining the magnitude of NO and CH_4 fluxes. However, since soil moisture is characterised by large microsite variation, results cannot be generalized to other forest edges. To better understand the environmental impact of N and C trace gas fluxes from forest edges, additional and long-term measurements in other forest edges are required. Given the stated importance of forest soils in global N and C budgets and continuing fragmentation trends, it is clear that more information on edge effects is required.

4. Edge effect on microbial community structure and N cycling

After: Remy E., Wuyts K., Verheyen K., Gundersen P., Boeckx P. Nitrogen cycling and microbial community structure at temperate forest edges (submitted).

Abstract

Due to forest fragmentation, forest edges have become dominant features in landscapes around the world. Forest edges are exposed to a different microclimate and to higher atmospheric nitrogen (N) deposition compared to the forest interior. It is still unclear how this elevated N deposition affects N cycling at temperate forest edges. In this study, the microbial community was mapped via the extraction of phospholipid fatty acids (PLFA) along edge-to-interior transects and amino sugars (AS) in forest edge (0 – 5 m) and interior (64 m) in two oak (*Quercus robur*) stands, two pine (*Pinus nigra*) stands and one spruce (*Picea sitchensis*) stand in northern Belgium and Denmark. Nitrogen mineralization, nitrification and immobilization rates were obtained via the *in situ* ^{15}N pool dilution technique in the forest edge and interior and linked to the microbial community structure. Furthermore, we assessed ^{15}N recovery in simulated throughfall via the ^{15}N tracing method in the edge and interior as a proxy for the long-term dynamics of the N cycle. Biomass of Gram+ bacteria was higher at the forest edges compared to the forest interiors and was associated to the observed higher mineralization rates. The oak stand was characterized by higher nitrification rates than the pine and spruce stands. In all forest types, the forest interior retained more N in the litter layer, while N was stored in deeper soil layers at the edge. Overall, our results indicated that the specific characteristics of the forest edge (atmospheric deposition, microclimate and soil physicochemical characteristics) increased microbial biomass, N turnover and storage capacity of soil layers beneath the litter layer and changed soil microbial community structure. Given the omnipresence of forest edges, more research should be conducted to validate our observations for other forest and soil types. Moreover, it is unclear how long these forest edges will be able to store additional N beneath the litter layer under ongoing high atmospheric deposition.

4.1. Introduction

Central and Western Europe are characterized by small forest remnants resulting from a long-term history of land-use change (Decocq et al. 2016; Hofmeister et al., 2013). Consequently, forest edges have become important features in the landscape (Harper et al., 2005). Forest edges differ substantially from forest interior zones, via changes in air and soil temperature, light availability, soil moisture and wind speed (e.g. Marchand and Houle 2006). Microclimatic gradients of soil temperature and moisture, among others, may cause altered decomposition and mineralization rates (Hobbie et al., 2007). Secondly, forest edges receive more atmospheric deposition, due to obstruction of the wind profile causing local advection and turbulent exchange (Draaijers, 1993). The edge effect on atmospheric deposition spans ca. 15 m to more than 100 m from the edge to the forest's interior and causes an up to five-fold increase in throughfall deposition (De Schrijver et al., 2007). The magnitude and depth of edge effects depend on several forest characteristics, including tree species, stand density and stand structure (Draaijers, 1993; De Schrijver et al., 1998; Devlaeminck et al., 2005; Wuyts et al. 2008a, 2008b, 2008c, 2009b).

Most forests of mid to high latitudes on the northern hemisphere were N limited until the 1950s, but due to a high atmospheric N load during the last decades this has changed considerably (Dupré et al., 2010). For example, in Europe, very high N deposition values (> 35 kg N ha^{-1} yr^{-1}) are observed in intensive livestock breeding areas (de Vries et al., 2011; MIRA 2011). Characteristics of N limitation are strong N recovery and efficient recycling of available N (Perakis et al., 2005). However, when forests become N saturated, excess N can be lost from the ecosystem via leaching and denitrification (Templer et al., 2012). Besides eutrophication, other harmful effects of increased N inputs include soil acidification, i.e. loss of exchangeable cations and the mobilization of aluminum and other potentially toxic metals (Wilpert et al., 2000), pollution of groundwater reserves (Dise et al., 2009) and biodiversity loss (De Schrijver et al., 2011). However, in forest edges, higher N deposition does not always lead to enhanced N losses, since Spangenberg and Kölling (2004) and Wuyts et al. (2011) found higher N deposition but lower inorganic N leaching in the first 30 m of forest edges of oak, birch, beech, spruce and pine monocultures compared to the forest interior. Moreover, Remy et al. (2016a) showed that gaseous N losses of nitric oxide (NO) were lower at the forest edge compared to the interior (see Chapter 3). Therefore, improved understanding of how ecosystem N pools and fluxes respond to increased N deposition is needed (Lu et al., 2011).

The ^{15}N tracing method is used to study the fate and recovery of N input (Schlesinger, 2009). By labelling N input with ^{15}N, the distribution of this N to the different ecosystem pools can be traced over time (Dörr et al., 2012). Furthermore, the ^{15}N pool dilution technique has been widely used to quantify gross N transformation rates (Dannenman et al., 2006; Staelens et al., 2012a). The principle is based on the dilution of a product pool that has been labelled with ^{15}N (Hart et al., 1994). However, many pool dilution experiments have been conducted via laboratory incubations, altering the *in situ* N transformation rates, as soil disturbance promotes gross N mineralization (Booth et al., 2006). Here, we used the *in situ* ^{15}N soil-labelling method, developed by Rütting et al. (2011), called the 'virtual soil core' injection, which enables the study of undisturbed soils with live roots and their associated microbial communities. Soils are only disrupted at sampling, insuring that soil temperature, water and gas exchange, as well as plant root and microbial activity remain under field conditions during the experiment.

The soil microbial community plays an essential role in the regulation of N cycling (Balser and Firestone, 2005). Scheu and Parkinson (1994) showed that fungi dominate in acid coniferous forest soils, although a shift to bacterial dominance may occur under the influence of high N deposition (Nilsson et al., 2007; Kjøller et al., 2012). Phospholipid fatty acids (PLFA) and amino sugars (AS) can both be used to determine the relative contribution of bacteria and fungi to the production of microbial derived organic matter (Frey, 2004; Nilsson et al., 2007). While PLFA are primarily derived from cell membranes, AS are released from cell wall biopolymers chitin and peptidoglycan (Zelles, 1999; Amelung, 2001). Lipid profiles can quantify presence and relative abundance of Gram- bacteria, Gram+ bacteria, actinobacteria and fungi in soil communities (Zogg et al., 1997). While PLFA are found in living organisms and decompose quickly after cell death (Zelles, 1999), AS are found in living and dead microbial biomass, reflecting historical microbial community changes and current community structure (Glaser et al., 2004). Of the more than 26 identified AS, only three occur in considerable amounts in soil, namely glucosamine, galactosamine and muramic acid (Griepentrog et al., 2014). Liang et al. (2008) stated that there is a lack of studies considering both PLFA and AS analysis to provide knowledge on the role of the microbial community in soil nutrient cycling.

The specific aims of this study were (i) to link soil microbial community structure along the edge-to-interior transects to N cycling rates, (ii) to quantify mineralization and nitrification rates, and N recovery in function of distance to the forest edge, and (iii) to interpret the ^{15}N recovery as an indicator for N retention, where low recoveries indicate an open N cycle. We

investigated the structure and abundance of the microbial community by extracting PLFA and AS from mineral soil in oak, pine and spruce stands, situated in agricultural landscapes in Belgium and Denmark. We used analytical pool dilution equations, originally developed by Kirkham and Bartholomew (1954), to quantify gross and net mineralization and nitrification rates in forest edges and interiors. Finally, we estimated N retention by following the fate of ^{15}N in simulated throughfall and measuring the percentage of added ^{15}N that was recovered over a period of 10 months in the organic and mineral soil layers. Due to the higher N deposition and contrasting microclimate at the forest edge, we hypothesized: (i) dominance of bacteria over fungi and (ii) higher mineralization and nitrification rates at the forest edge; consequently (iii) higher nitrification rates would be expected to lead to higher N losses and thus lower ^{15}N recovery in the forest edge.

4.2. Material and methods

4.2.1. Study sites

The study was performed in the oak stand near Ravels (Qr2), in the pine stand in Poppel (Pn2), both situated in the province of Antwerp (Belgium) and in the spruce stand in Sonder Omme, Denmark (Ps, Table 1.1). The microbial community was identified in two more forest stands, the oak stand (Qr1) in Wortegem and the pine stand (Pn1) in Zedelgem, both in the province of West-Flanders (Belgium). The mean annual air temperature and precipitation in 1981 - 2010 are 10.5°C and 800 mm in Belgium and 7.4°C and 900 mm on the peninsula of Jutland (Denmark; data obtained from the nearest weather station operated by the Royal Meteorological Institute of Belgium and the Danish Meteorological Institute respectively for the Belgian and Danish forests). Monospecific forest stands of pedunculate oak (*Quercus robur* L.), Corsican pine (*P. nigra* ssp. *laricio* Maire) and Sitka spruce (*Picea sitchensis* (Bong) Carr.) were selected with similar soil type, stand history and edge orientation (Table 1.1). All forests are even-aged monocultures growing on acid, quartz-dominated Podzols. Previous land use was heathland before the afforestation last century. The considered forest edges are facing the locally prevailing wind direction (west to southwest), which creates the steepest throughfall deposition gradients (Draaijers et al., 1988). Yearly N throughfall deposition fluxes are available from previous studies in the same forest stands at exactly the same distances from the edge (Wuyts et al., 2008b, 2011; Ginzburg, 2014, Table 1.1). Average yearly N leaching fluxes were 27 kg ha^{-1}, 17 kg ha^{-1} and 9 kg ha^{-1}, respectively at the edge (0 – 20 m) of Qr2, Pn2 and Ps. Average yearly N leaching in the interior amounted to 25 kg ha^{-1}, 16 kg ha^{-1} and 5 kg ha^{-1}, respectively in Qr2, Pn2 and Ps.

4.2.2. Soil microbial community

4.2.2.1. Sampling and extraction

For the PLFA analysis, three replicate samples in all five forests were taken (September 2015) with a soil auger from the upper 10 cm of the mineral soil (Fig. A-I), at the forest edge (0 - 5 m) and at 16, 64 and 128 m from the forest edge. Soil samples were frozen until further analysis. Phospholipid fatty acids (PLFA) were extracted following a procedure explained in detail by Moeskops et al. (2010). Eight g of mineral soil from each sample was sieved (2mm) in order to homogenize and remove the root fragments and stones. This sieved soil was subjected to different extraction procedures for three days. On the first day, total lipids were separated from other soil components using a multi-phase extraction mixture of P buffer (0.1 M, pH 7.0, mixture of K_2HPO_4 and KH_2PO_4), chloroform ($CHCl_3$) and methanol (CH_3OH) in volume ratios of 0.8:1:2, in a separation funnel. On the second day, the extracted lipids were fractionated into neutral, glyco- and phospholipids using silica Solid Phase Extraction (SPE) cartridges (Chromabond, MachereyeNagel GmbH, Germany), after which only phospholipids were kept for further analysis. The separated PLFA were transformed into fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. After drying the FAMEs under N_2 gas, the FAMEs were re-dissolved in hexane containing nonadecanoic acid methyl ester (C19:0) as an internal standard FAME. Finally, individual FAMEs were identified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS) on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Interscience BVBA, Belgium) in electron ionization mode. The PLFA i15:0, a15:0, 15:0, i16:0, 17:0, i17:0 and a17:0 were indicators of Gram+ bacteria, while PLFA 16:1w7c, 16:1w9c, cy17:0 and cy19:0 were indicators of Gram- bacteria. Fungal PLFA were 18:1w9c, 18:2w6c and 18:3w6c.

Amino sugar analysis was performed on three replicate samples of the 0 - 10 cm mineral soil at the forest edge (0 – 5 m) and at the forest interior (64 m) collected in September 2015. Extraction of amino sugars from bulk soil samples was adapted from the method described by Zhang et al. (1998) and Bodé et al. (2009). Mineral soil (0.5 g) was hydrolyzed by adding 10 ml 6 M HCl and an internal standard (myo-inositol) and subsequently heated for 8 h at 105 °C. Samples were filtered over glass fiber filters (GF/C, Whatman, Dassel, Germany) and the filtrate was evaporated to dryness at 40–45 °C under reduced pressure to remove HCl. Dried filtrate was redissolved in Milli-Q water (Direct-Q 3 System, Millipore, Billerica, MA, USA), transferred in a 2 ml tube (Eppendorf, Hamburg, Germany) and centrifuged. The supernatant was added onto a cation exchange resin (AG 50W-X8, Bio-Rad Laboratories, Hercules, CA, USA). After rinsing the resin with Milli-Q water to remove neutral and

negatively charged compounds, the fraction containing amino sugars was eluted with 0.5 M HCl and again evaporated to dryness to remove HCl. Dried amino sugars were redissolved in Milli-Q water and transferred in a 2 ml tube. After desiccation using a centrifugal vacuum concentrator (SpeedVac, Thermo Scientific, Langenselbold, Germany), samples were stored at -18 °C until analysis. Compound-specific stable isotope analysis of amino sugar extracts was performed according to the method described by Bodé et al. (2009). Therefore, we used a high pressure liquid chromatography (HPLC) system existing of an autosampler (Surveyor Autosampler Plus, Thermo Electron, Germany) and a HPLC pump (Surveyor MS-Pump Plus, Thermo Electron, Germany) with an analytical anion-exchange column (PA20 CarboPac, 3 9 150 mm, 6.5 lm) that was coupled through a wet oxidation interface (LC Isolink, Thermo Electron, Germany) to an IRMS (DELTAPLUS XP, Thermo Electron, Germany). The AS muramic acid is derived exclusively from peptidoglycan of bacterial cell walls, while glucosamine is present in fungal and bacterial cell walls, respectively as part of chitin and peptidoglycan. Galactosamine is predominantly from bacterial origin (Frey et al., 2004). Ratios of glucosamine to muramic acid (GluMur) and of glucosamine to galactosamine (GluGal) are used as indicators of the relative contribution of fungi versus bacteria to the microbial community structure (Amelung, 2001).

4.2.2.2. Statistical analysis

All statistical analyses were performed using the software package R (version 3.3.1.) including package lme4 (Bates et al., 2015). We tested if variations in PLFA and AS concentrations and the fungal to bacterial ratios were associated with distance to the forest edge and the examined forest type via linear mixed effect models. We used the following variables as response variables: total PLFA concentrations, total fungal PLFA, total bacterial PLFA, ratio of fungal to bacterial PLFA, Gram+ PLFA, Gram- PLFA, total AS, GluMur and GluGal. Predictor variables were distance to the forest edge, forest type (oak, pine, spruce) and the interaction of distance to the forest edge and forest type. Firstly, the need of a linear mixed-effect model, including the forest location (five stand locations) as a random factor, was tested for each response variable. In this way, the non-independence of samples from the same forest has been taken into account. The linear mixed-effect model was compared with a linear model with the same predictor variables. The appropriate model was chosen based on the lowest AIC (Akaike Information Criterion) value. The contribution of each predictor variable to the model was tested against the null model with one-way analysis of variance (ANOVA). Normality, homoscedasticity and the relationship between the fitted values and the residuals of each model was checked via diagnostic plots.

Next, we used Spearman rank correlation analyses (r_s = correlation coefficient) to examine correlations between PLFA and AS concentration (total PLFA, fungal PLFA, bacterial PLFA, ratio of fungal to bacterial PLFA, total AS, GluMur and GluGal) and environmental and soil physicochemical variables (soil moisture, soil temperature, N deposition, C/N ratio of forest floor and mineral soil and pH-KCl of mineral soil). Soil moisture (TDR probe CS 625-L, Campbell Scientific, United Kingdom) at the edge, 16 m, 32 m and 64 m and temperature (iButton DS1921G, Fondriest Environmental, USA) measurements at the edge, 16 m, 64 m and 128 m from the edge were executed every 2 hours at a depth of 5 cm from November 2013 until November 2015. Nitrogen deposition, soil and forest floor C/N ratios and soil pH values were extracted from previous studies by Wuyts et al. (2008b, 2013) and Ginzburg (2014) in the same forest stands.

4.2.3. Nitrogen transformation in mineral soil

4.2.3.1. Plot installation and N addition

In Qr1, Pn1 and Ps, ten 1 x 1 m² plots were selected at the edge (0 – 5 m) and interior (64 m) with an inter-distance of 8 m. One week prior to ^{15}N addition, the fresh litter layer (L) and fermentation and humus layer (FH) were carefully removed over an area of 40 x 40 cm². Two separate nylon meshes (12 x 20 cm², 1 mm mesh size), indicating the two injection and sampling locations (§ 4.2.3.2.) were put on top of the mineral soil before putting back the FH and L layer. The mesh allowed distinguishing the top of the mineral layer (Fig. A-I) in the following ^{15}N label injection and soil sampling steps. The ^{15}N treatments were applied to five sites each, both at the edge and interior. Each treatment consisted of a water solution with ammonium (NH_4^+) and nitrate (NO_3^-) in which one of the N moieties was labelled with ^{15}N at 99 atom % excess. The ^{15}N was applied as $^{15}NH_4Cl$ and $Na^{15}NO_3$ and added concentrations were based on actual measured concentrations of NH_4^+ and NO_3^- in soil, one week prior to the injections. Briefly, a subsample of 5 g sieved fresh mineral soil was extracted with 10 ml 1 M KCl, shaken for 1 h (150 rpm), and filtered (Schleicher & Schuell Microscience 598 ½) prior to analysis of NH_4^+ and NO_3^- concentrations. Ammonium was determined colorimetrically by the salycilate-nitroprusside method (Mulvaney, 1996) on an auto-analyzer (AA3, Bran & Luebbe, Germany). Nitrate was determined colorimetrically using the same auto-analyzer after reduction of NO_3^- to nitrite (NO_2^-) in a Cu-Cd column, followed by the reaction of NO_2^- with N-1-napthylethylenediamine to produce a chromophore. In Qr1, Pn1 and Ps 30.9 mg NH_4-N l^{-1} and 23.6 mg NO_3-N l^{-1}, 32.5 mg NH_4-N l^{-1} and 2.7 mg NO_3-N l^{-1} and 43.6 mg NH_4-N l^{-1} and 38.3 mg NO_3-N l^{-1} was added, respectively. To assure an even

distribution of the applied N in the mineral topsoil (0 – 10 cm), the solutions were injected four times using a holder consisting of four 1.5 ml needles (10 cm length), spaced 3 cm from each other, into the soil surface covered by the nylon mesh, resulting in a grid of 16 injection points. Short-term experiments of 2 days were used to avoid remineralization of immobilized ^{15}N (Takahashi, 2001). Moreover, ^{15}N was injected in a larger area than the area that would be sampled, to avoid ^{15}N dilution from non-labelled soil, as recommended by Rütting et al. (2011). The applied ^{15}N label was homogenously injected into the virtual soil cores. The ^{15}N treatments ($^{15}NH_4^+$ and $^{15}NO_3^-$) were applied concurrently, after which the locations were immediately covered with the nylon mesh, FH and L layers.

4.2.3.2. Sampling and chemical analysis

Before soil sampling, the L and FH layers and the nylon mesh were removed. The ^{15}N labelled mineral soil was sampled with a PVC tube (5 cm inner diameter) that was pushed 10 cm into the soil in the middle of the soil surface injected with^{15}N. The 0-10 cm mineral soil was sampled 15 min and 2 days after label injection. The experiment was conducted during clear, sunny days in June. Care was taken to keep the same time lag between soil injection, sampling and processing (within 1.5 h after the sampling) for each of the five spatial replicates per location (edge-interior), treatment and time step. A subsample of 60 g fresh soil was sieved, extracted with 120 ml 1 M KCl, shaken for 1 h (150 rpm) and filtered (Schleicher & Schuell Microscience 598 ½) prior to the analysis of ^{15}N contents of NH_4^+ and NO_3^-. The ^{15}N contents of NH_4^+ and NO_3^- were analysed after conversion to N_2O using a trace gas preparation unit (ANCA-TGII, Sercon, UK) coupled to an IRMS (20-20, Sercon, UK). Ammonium was converted by adding MgO to soil extracts and absorbing NH_3 into H_2SO_4, after which N_2O was produced by reaction with NaOBr (Saghir et al., 1993). Nitrate was reduced by Cd-Cu at pH 4.7 to produce nitrite and NH_2OH as intermediates of N_2O (Stevens et al., 1998). Nitrate concentrations were very low in the spruce forest Ps, therefore conversions to N_2O were done by bacterial denitrification, as described by Xue et al. (2013). This method allows for the determination of $\delta^{15}N$ of N_2O produced from the conversion of NO_3^- by denitrifying bacteria, which naturally lack N_2O-reductase activity (Xue et al., 2010).

Gross mineralisation (m) was calculated via the dilution of $^{15}NH_4^+$ (Eq. 4.1) and gross nitrification (n) via the dilution of $^{15}NO_3^-$ (Eq. 4.2), taken from Griffin (2007), but based on the original equation from Kirkham and Bartholomew (1954).

$$m\ (mg\ N\ kg^{-1}\ soil\ d^{-1}) = \frac{[NH_4^+]_0 - [NH_4^+]_t}{t}\ x\ \frac{\log(\frac{APE_0}{APE_t})}{\log[NH_4^+]_0/[NH_4^+]_t} \qquad \text{(Eq. 4.1)}$$

$$n\ (mg\ N\ kg^{-1} soil\ d^{-1}) = \frac{[NO_3^-]_0 - [NO_3^-]_t}{t}\ x\ \frac{\log(\frac{APE_0}{APE_t})}{\log[NO_3^-]_0/[NO_3^-]_t} \quad \text{(Eq. 4.2)}$$

where $[NH_4^+]_0$ and $[NH_4^+]_t$ are soil NH_4^+ concentrations at time zero (15 min. and time t (2 days), $[NO_3^-]_0$ and $[NO_3^-]_t$ are soil NO_3^- concentrations at time zero and time t and APE_0 and APE_t are atom percent excess (APE) of the respective N species at time zero and t, respectively. Immobilisation rates (i) were calculated based on Eq. (4.3) and (4.4), respectively for NH_4^+ and NO_3^-.

$$i_{NH_4^+} = \frac{m - ([NH_4^+]_t - [NH_4^+]_0)}{t} \quad \text{(Eq. 4.3)}$$

$$i_{NO_3^-} = \frac{n - ([NO_3^-]_t - [NO_3^-]_0)}{t} \quad \text{(Eq. 4.4)}$$

Net mineralisation and nitrification rates were obtained by subtracting immobilisation from gross mineralisation and nitrification rates. The remaining soil was weighed and dried for 48 h at 105 °C to determine soil moisture content.

4.2.3.3. Statistical analysis

Firstly, the need of a linear mixed effect model, including the forest location (five stand locations) as a random factor, was tested for each response variable (mineralization, nitrification and immobilization). This linear mixed effect model was compared with a linear model, where the latter had the lowest AIC (Akaike Information Criterion) value. Two-way analysis of variance (ANOVA) was used to test the influence of edge proximity (edge, interior), forest type (oak, pine, spruce) and their interaction on mineralization, nitrification and immobilization, followed by a post hoc Tukey's honestly significant difference (HSD) test with significance for $p < 0.05$. We used Spearman rank correlation analyses to examine correlations between gross and net mineralization rate and environmental and soil physicochemical variables (soil moisture, soil temperature, N deposition, C/N ratios of forest floor and mineral soil, and pH-KCl of mineral soil) on the one hand and PLFA and AS concentration (total PLFA, fungal PLFA, bacterial PLFA, ratio of fungal to bacterial PLFA, Gram+, Gram-, total AS, GluMur and GluGal) on the other hand. The same correlations were checked for the gross and net nitrification and immobilization rates.

4.2.4. Nitrogen recovery

4.2.4.1. Plot installation and N addition

Per forest stand, six 1 x 1 m² plots with an inter-distance of 10 m were selected at the edge (0 – 5 m) and in the forest interior (64 m). The application of ^{15}N was done in summer (August 2014) using a hand sprayer (Gardena comfort hand sprayer 1.25 l, Germany). Per stand, three plots received $^{15}NH_4Cl$ and three plots $Na^{15}NO_3$ (with ^{15}N at 99 atom % excess). The total added ^{15}N amount was 30 mg m^{-2}. First 500 ml of the ^{15}N-solution was added on top of the forest floor of a plot, followed by 500 ml of distilled water to insure a swift infiltration of the ^{15}N-solution.

4.2.4.2. Sampling and chemical analysis

Litter (L), fermentation and humus (FH) and mineral soil (Fig. A-I) sampling took place one day (T1), one month (T2) and ten months (T3) after the first ^{15}N addition. In each plot, two forest floor samples were collected using a wooden frame (20 x 20 cm^2). The litter layer was sampled separately from the fermentation and humus layer. Where the forest floor was removed, two samples of two mineral soil layers (0-10 cm, MS10 and 10-20 cm, MS20) were sampled using sharpened PVC tubes (5 cm inner diameter). The litter and FH samples were dried at 65°C for 48 h and milled (ZM1, Retsch, Germany), while soil samples were dried at 40 °C for 48 h and thereafter ground by a planetary ball mill (PM400, Retsch, Germany) for total N and ^{15}N analysis by an elemental analyzer (EA) (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (IRMS) (20-20, SerCon, UK). Natural abundance of ^{15}N in mineral soil had previously been determined for each forest (unpublished data). Percent of ^{15}N tracer recovery was calculated based on a ^{15}N mass balance (Nadelhoffer et al., 1999):

$$^{15}N_{rec}\ (\%) = \frac{m_f\ (atom\%\ ^{15}N_f - atom\%\ ^{15}N_i)}{m_t (atom\%\ ^{15}N_t)}\ x\ 100 \qquad \text{(Eq. 4.5)}$$

where $^{15}N_{rec}$ = percent of ^{15}N tracer recovered; m_f = N pool of each ecosystem compartment (t N ha^{-1}); atom% $^{15}N_f$ = atom percent ^{15}N in the N pool; atom% $^{15}N_i$ = atom percent ^{15}N in the reference N pool (i.e. natural ^{15}N abundance); m_t = the mass of tracer applied and atom% $^{15}N_t$ = atom percent ^{15}N in excess in the added tracer. Atom percent excess values indicate the abundance of a stable nuclide in a sample expressed in terms of the excess, in atom percent, over that naturally present (= 0.37 at%).

4.2.4.3. Statistical analysis

A linear mixed-effect model was used to assess the effect of the discrete predictor variables: edge proximity (edge, interior), time of sampling (1 day, 1 month, 10 months), layer (L, FH, mineral soil) and their interactions on ^{15}N recovery. The treatments of $^{15}NH_4Cl$ and $Na^{15}NO_3$ were analysed separately. The model comprised a random term, i.e. plot, to account for dependency between samples taken from the same plot. We included the function varIdent into the model, to correct the residuals for heteroscedasticity. The significance of the different predictor variables was assessed by analysis of variance (ANOVA) with a significance level of $p < 0.05$. The relationship between the fitted values and the residuals of each model was checked to ensure normality and homoscedasticity. We used Spearman rank correlation analyses to examine correlations between ^{15}N recovery and environmental and soil physicochemical variables (soil moisture, soil temperature, N deposition, C/N ratios of forest floor and mineral soil, and pH-KCl of mineral soil), PLFA and AS concentration (total PLFA, fungal PLFA, bacterial PLFA, ratio of fungal to bacterial PLFA, Gram+, Gram-, total AS, GluMur and GluGal).

4.3. Results

4.3.1. Soil microbial community

4.3.1.1. Phospholipid fatty acids

Total PLFA concentrations were on average higher in the oak stands (11.6 ± 8.6 µg g^{-1} soil) than in the pine stands (5.3 ± 0.6 µg g^{-1} soil) and the spruce stand (3.3 ± 0.8 µg g^{-1} soil). Distance to the forest edge, forest type and the interaction of distance to the forest edge and forest type significantly affected total PLFA concentrations (Table 4.1). Total PLFA decreased with distance to the edge in the oak and pine stands. In Qr2, Pn1 and Pn2, total PLFA concentrations were 63 % higher at the forest edge, while in Qr1 total PLFA concentrations were 3 times higher at the forest edge compared to the interior. Fungal PLFA concentrations were only influenced by forest type, being higher in the oak stands than in the pine and spruce stands. Bacterial PLFA (Gram+) were influenced by distance to the forest edge, being higher at the edge. In the oak stands, Gram+ PLFA concentrations were 3 (Qr1) and 2 (Qr2) times higher at the forest edge compared to the interior. In the pine stands, Gram+ PLFA concentrations were 65 % higher at the forest edge, while Gram+ PLFA concentrations were similar for edge and interior in the spruce stand. Fig. 4.1 shows the Gram+ PLFA concentrations for the oak, pine and spruce stands, where the warmer and

more N-rich edge plots can be distinguished from the interior plots. Gram- bacteria were significantly influenced by the interaction of distance to the forest edge and forest type. With increasing edge distance, the PLFA concentration of Gram- bacteria decreased in the oak stands and stayed rather constant in the pine and spruce stands. The ratio of fungal to bacterial PLFA was not significantly different between forest edge and interior. The correlations between the microbial community and the environmental and soil physicochemical variables can be seen in Fig. 4.2a. The correlations among the environmental and soil physicochemical variables can be seen in Table 4.2. Total and bacterial PLFA concentrations were negatively correlated to the C/N ratio of the forest floor (L and FH, with respectively r_s = -0.75, $p < 0.001$, n = 20 and r_s = -0.68, p = 0.001, n = 20) and positively to the soil temperature (respectively r_s = 0.46, $p < 0.05$, n = 20 and r_s = 0.49, $p < 0.05$, n = 20) and pH of mineral soil (respectively r_s = 0.68, $p < 0.001$, n = 20 and r_s = 0.70, $p < 0.001$, n = 20). Fungal PLFA concentrations were negatively correlated to the C/N ratio of the forest floor (r_s = -0.83, $p < 0.001$, n = 20) and positively to the pH of mineral soil (r_s = 0.55, $p < 0.05$, n = 20). The ratio of fungal to bacterial PLFA was negatively correlated to N deposition (r_s = -0.47, $p < 0.05$, n = 20) and positively to soil moisture (r_s = 0.78, $p < 0.01$, n = 12). Covariation of N deposition and C/N ratio of mineral soil (Table 4.2) probably led to a negative correlation with C/N ratio of the forest floor (r_s = -0.58, $p < 0.01$, n = 20) and C/N ratio of mineral soil (r_s = -0.46, $p < 0.05$, n = 20). An overview of the bacterial, Gram+, Gram- and fungal PLFA concentrations and their ratio can be found in Table A-II.

4.3.1.2. Amino sugars

Total AS concentration was on average 1500 ± 687 µg g^{-1} soil in the oak stands and differed from the concentrations in the pine stands (675 ± 235 µg g^{-1} soil) and the spruce stand (337 ± 106 µg g^{-1} soil). Total AS concentration was not affected by distance to the forest edge, but differed by forest type (Table 4.1). An overview of the concentrations of glucosamine, muramic acid and galactosamine and the ratios of GluMur and GluGal can be found in Table A-II. Total AS concentration was negatively correlated to the C/N ratio of the forest floor (r_s = -0.93, $p < 0.001$, n = 10). The ratio of GluMur (fungi/bacteria) was positively correlated to the pH of mineral soil (r_s = 0.77, $p < 0.01$, n = 10) and the ratio of GluGal (fungi/bacteria) was positively correlated to N deposition (r_s = 0.68, $p < 0.05$, n = 10).

Table 4.1: Effects of distance to the forest edge and forest type on phospholipid fatty acid (PLFA) and aminosugar (AS) concentration. Bold values are significant ($p < 0.05$).

PLFA	Distance to forest edge		Forest type		Interaction		R^2
Total	**< 0.05**	↓	**< 0.05**	o > p, s	**< 0.05**	→	0.55
Fungi	0.999		**< 0.05**	o > p, s	0.999		0.34
Bacteria	**< 0.05**	↓	**< 0.05**	o > p, s	**< 0.01**	→	0.62
Gram+	**< 0.01**	↓	**< 0.05**	o > p, s	**< 0.01**	→	0.66
Gram-	0.071		**< 0.05**	o > p, s	**< 0.05**	→	0.59
Ratio Fungi/Bacteria	0.075		**< 0.01**	o > p, s	0.505		0.55
AS							
Total	0.199		**< 0.05**	o > p, s	**< 0.01**	→	0.83
Ratio GluMur	0.611		0.208		0.768		-
Ratio GluGal	0.180		0.290		0.867		-

Glu = Glucosamine, Mur = Muramic acid, Gal = Galactosamine. R^2 is the coefficient of determination, indicating the proportion of variation explained by the model. The forest-type effect is specified, where o = oak, p = pine and s = spruce. The arrow (↓) indicates that values decrease with distance to the edge. The neutral interaction (→) indicates that the edge effect differs between forest types.

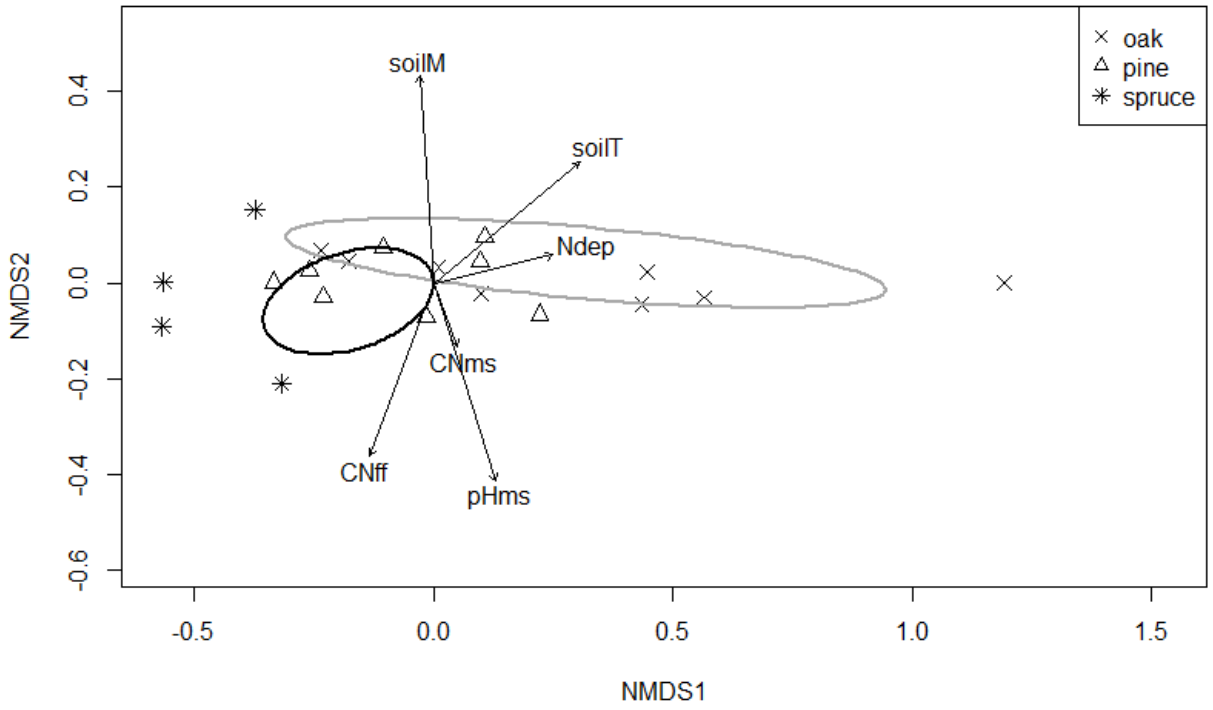

Fig. 4.1: Non-metric multidimensional scaling (NMDS) ordination diagram (with Bray-Curtis dissimilarity) of the PLFA concentrations related to Gram+ bacteria (i15:0, a15:0, 15:0, i16:0, 17:0, i17:0 and a17:0) along the edge-to-interior transects for the oak, the pine and the spruce stands with fitted environmental and soil physicochemical variables. The grey ellipse indicates the edge plots and the black ellipse the interior plots (= 128 m). soilM = soil moisture at a depth of 5 cm, soilT = soil temperature at a depth of 5 cm, Ndep = N deposition (kg N ha^{-1} yr^{-1}), CNff = C/N ratio of the forest floor (L + FH layer), CNms = C/N ratio of the mineral soil, pHms = pH-KCl of the mineral soil.

Table 4.2: Spearman rank correlation coefficients (r_s) between environmental and soil physicochemical variables.

r_s	soilM	soilT	Ndep	CNff	CNms
soilM	-				
soilT	-0.047	-			
Ndep	-0.164	0.627 **	-		
CNff	-0.421	-0.398	0.121	-	
CNms	-0.413	0.296	0.717 ***	0.412	-
pHms	-0.704 *	0.204	-0.287	-0.255	-0.277

soilM = soil moisture at a depth of 5 cm, soilT = soil temperature at a depth of 5 cm, Ndep = N deposition (kg N ha^{-1} yr^{-1}), CNff = C/N ratio of the forest floor (L + FH layer), CNms = C/N ratio of the mineral soil, pHms = pH-KCl of the mineral soil.*p < 0.05, ** p < 0.01, *** p < 0.001.

4.3.2. Nitrogen transformation

4.3.2.1. Mineralization

Gross mineralization rates were on average 0.88 ± 0.96 mg N kg^{-1} soil d^{-1} in the oak stand (Qr2), 0.84 ± 0.91 mg N kg^{-1} soil d^{-1} in the pine stand (Pn2) and 0.10 ± 0.03 mg N kg^{-1} soil d^{-1} in the spruce stand. Gross mineralization rates were higher in the forest edge than in the forest interior and differed between forest types (Table 4.3). In the oak and pine stands, mineralization rates were on average 7 times higher at the forest edge, while in the spruce forest mineralization rates were on average 66 % higher at the forest edge compared to the interior. The spruce stand had lower gross mineralization rates than the oak and the pine stands. Ammonium immobilization and net mineralization differed neither between edge and interior, nor between forest types (Table 4.3). The correlations between gross mineralization, NH_4^+ immobilization and net mineralization and the environmental and soil physicochemical variables can be seen in Fig. 4.2b. Gross mineralization rates were positively correlated with the soil temperature (r_s = 0.94, p < 0.05, n = 6). Correlations between the soil microbial community and N transformation rates can be seen in Fig. 4.2d. Gross mineralization rates were positively correlated with total, fungal and bacterial PLFA concentrations (r_s = 0.94, p < 0.05, n = 6).

4.3.2.2. Nitrification

Gross nitrification rates were on average 0.53 ± 0.05 mg N kg^{-1} soil d^{-1} in the oak stand (Qr2), 0.10 ± 0.05 mg N kg^{-1} soil d^{-1} in the pine stand (Pn2) and 0.02 ± 0.006 mg N kg^{-1} soil d^{-1} in the spruce stand. Gross nitrification rates did not differ significantly between forest edge and interior, but differed between forest types (Table 4.3). Nitrate immobilization rates differed neither between forest edge and interior, nor between forest types. Net nitrification was not influenced by edge proximity, but differed between forest types (Table 4.3). The oak stand was characterised by higher gross and net nitrification rates than the pine and spruce stands. The correlations between gross nitrification, NO_3^- immobilization and net nitrification and the environmental and soil physicochemical variables can be seen in Fig. 4.2b. Gross and net nitrification rates were negatively correlated with the C/N ratio of the forest floor (L and FH layer) (r_s = -0.88, $p < 0.05$, n = 6). Gross nitrification rates were positively correlated with total AS concentration (r_s = 0.94, $p < 0.05$, n = 6, Fig. 4.2d).

Table 4.3: Gross and net mineralization, NH_4^+ and NO_3^- immobilization, and gross and net nitrification rates (mg N kg^{-1} soil d^{-1}) in the oak (Qr2), pine (Pn2) and spruce stand. Standard errors are presented between brackets (n = 5). Bold values indicate a significant edge effect ($p < 0.05$), different letters indicate significant differences between forest types ($p < 0.05$).

	Oak				Pine				Spruce			
N transformation	Edge		Interior		Edge		Interior		Edge		Interior	
Gross mineralization	**1.56**	(1.22)a	**0.20**	(0.03)a	**1.49**	(1.01)a	**0.20**	(0.07)a	**0.12**	(0.04)b	**0.07**	(0.01)b
NH_4^+ immobilization	0.73	(0.61)	-0.21	(0.15)	-0.58	(0.89)	0.28	(0.12)	0.28	(0.20)	-0.19	(0.27)
Net mineralization	0.83	(0.62)	0.41	(0.15)	2.07	(1.88)	-0.08	(0.08)	-0.15	(0.17)	0.27	(0.27)
Gross nitrification	0.56	(0.14)a	0.49	(0.22)a	0.06	(0.02)b	0.14	(0.26)b	0.01	(0.002)b	0.02	(0.01)b
NO_3^- immobilization	-0.13	(0.22)	-0.23	(0.33)	-0.07	(0.06)	-0.16	(0.14)	0.01	(0.03)	0.03	(0.01)
Net nitrification	0.69	(0.35)a	0.71	(0.23)a	0.13	(0.05)b	0.30	(0.22)b	0.004	(0.03)b	-0.01	(0.01)b

Edge = 0 – 5 m, Interior = 64 m

4.3.3. Nitrogen recovery

In the oak stand (Qr2), recovery of $^{15}NH_4^+$ in the litter layer was consistently lower (on average 60 %) at the edge compared to the interior ($p < 0.01$, Table 4.4), while there were no significant differences between edge and interior in the other soil layers. Recovery of $^{15}NO_3^-$ after 1 day was high in the litter layer (i.e. 69 ± 23 %), but lowered after 1 month to on average 13 ± 8 % both at the forest edge and forest interior. Recovery of $^{15}NO_3^-$ was higher in the forest edge compared to the interior in the fermentation and humus (FH) layer (on average 76 %, $p < 0.01$) and in both mineral soil layers (respectively 3 times higher at the forest edge for MS10 and 9 times higher at the forest edge for MS20, $p < 0.01$, Table 4.3). Due to low enrichments and high variability in mineral soil, average $^{15}N_{rec}$ values were sometimes negative.

In the litter layer of the pine stand, recovery of $^{15}NH_4^+$ lowered over time ($p < 0.001$), while in the FH layer the edge effect was significant ($p < 0.05$, Table 4.4). After 1 day and 1 month, recovery of $^{15}NH_4^+$ was 2 times higher in the forest edge than in the interior, but after 10 months the recovery was 64 % higher in the FH layer at the forest edge. In the mineral soil layers, recovery of $^{15}NH_4^+$ was lower at the forest edge compared to the forest interior after 1 day and 1 month (2 times lower at the forest edge, $p = 0.001$ and $p < 0.01$, respectively for MS10 and MS20), but equalled after 10 months. The recovery of $^{15}NO_3^-$ in the litter layer differed between edge and interior in function of time ($p < 0.05$, Table 4.4). After 1 day, recovery of $^{15}NO_3^-$ was 60 % higher at the forest edge compared to the interior, but after 1 month, recovery of $^{15}NO_3^-$ was 16 % lower at the edge and after 10 months it was 63 % lower at the forest edge. In the other soil layers, there were no significant edge effects on the recovery of $^{15}NO_3^-$. Due to high enrichments and variability, average $^{15}N_{rec}$ values were sometimes > 100 % in the litter layer of the pine and spruce stand.

In the spruce stand there was no significant effect of edge proximity on the recovery of $^{15}NH_4^+$ ($p > 0.05$). For $^{15}NO_3^-$, recovery was significantly lower in the forest edge than in the interior in the FH layer ($p < 0.01$, Table 4.4). After 1 day, recovery of $^{15}NO_3^-$ was 68 % lower at the edge, after 1 month, recovery of $^{15}NO_3^-$ was 38 % lower at the edge and after 10 months recovery of $^{15}NO_3^-$ equalled between edge and interior. An overview of all mean $^{15}N_{rec}$ (%) at the different times of sampling at the edge and interior of the oak, pine and spruce forest can be found in Table A-III. Recovery of ^{15}N lowered throughout the different soil layers, i.e. from litter to mineral soil, being very low in the mineral soil layers. There were no significant correlations between the total recovery of $^{15}NH_4^+$ and $^{15}NO_3^-$ and

Table 4.4: a) Mean $^{15}N_{rec}$ (%) values after 10 months (T3) at the edge and interior of the oak (Qr2), pine (Pn2) and spruce stand. Standard errors are presented between brackets (n = 6). b) Effects of distance to the forest edge, layer and time on the $^{15}N_{rec}$ (%) values in the oak, pine and spruce stand.

a		Oak				Pine				Spruce			
Layer	Treatment	Edge		Interior		Edge		Interior		Edge		Interior	
L	$^{15}NH_4^+$	9.8	(2.9)	23.5	(15.5)	31.4	(16.5)	29.5	(19.6)	119.8	(45.4)	42.7	(8.1)
	$^{15}NO_3^-$	10.6	(5.0)	5.3	(4.8)	44.1	(30.9)	120.6	(28.4)	66.2	(28.2)	52.9	(20.6)
FH	$^{15}NH_4^+$	25.9	(13.8)	10.2	(5.5)	29.8	(30.3)	18.1	(12.3)	16.3	(6.9)	30.7	(16.7)
	$^{15}NO_3^-$	17.7	(9.8)	7.6	(7.7)	35.7	(28.4)	35.2	(11.9)	25.9	(8.9)	27.2	(11.3)
MS10	$^{15}NH_4^+$	2.1	(12.1)	-4.9	(1.7)	2.3	(1.2)	3.7	(1.2)	10.2	(6.4)	5.8	(14.1)
	$^{15}NO_3^-$	5.2	(2.8)	-3.2	(3.6)	-0.6	(1.0)	1.3	(1.9)	5.0	(12.9)	5.8	(13.3)
MS20	$^{15}NH_4^+$	1.5	(8.7)	-2.6	(2.3)	-1.2	(2.2)	2.5	(3.6)	0.4	(1.8)	2.8	(5.5)
	$^{15}NO_3^-$	8.2	(5.1)	-0.9	(1.8)	-0.8	(2.1)	7.9	(9.7)	3.5	(5.0)	0.1	(2.5)

b						
Forest type	Treatment	Distance to edge	Layer	Time	Distance to edge : Layer	Distance to edge : Time
Oak	$^{15}NH_4^+$	0.054	**< 0.001**	**< 0.05**	**< 0.001**	0.440
	$^{15}NO_3^-$	**< 0.001**	**< 0.001**	**< 0.01**	0.220	0.600
Pine	$^{15}NH_4^+$	**< 0.05**	**< 0.001**	0.053	**< 0.001**	**< 0.001**
	$^{15}NO_3^-$	**< 0.001**	**< 0.001**	**< 0.01**	**< 0.001**	0.740
Spruce	$^{15}NH_4^+$	0.240	**< 0.001**	**< 0.001**	**< 0.001**	0.300
	$^{15}NO_3^-$	**< 0.01**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.01**

L = Litter, FH = fermentation and humus layer, MS10 = Mineral soil 0 - 10 cm, MS20 = Mineral soil 10 - 20 cm; Edge = 0 – 5 m, Interior = 64 m.

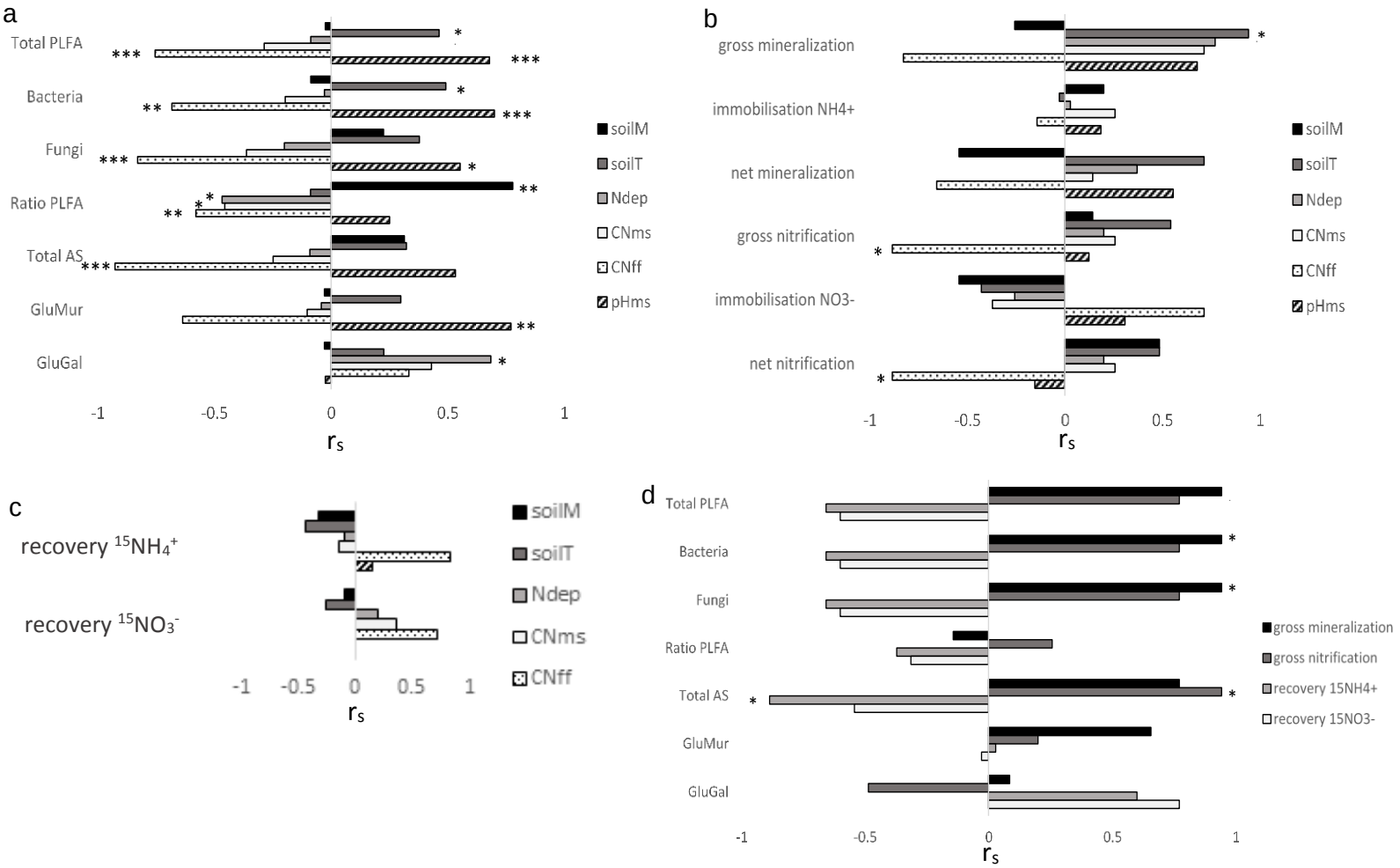

Fig. 4.2: Spearman rank correlations between a) the soil microbial community structure and environmental and soil physicochemical variables, b) N transformation rates and environmental and soil physicochemical variables, c) total ^{15}N recovery and environmental and soil physicochemical variables and d) the soil microbial community structure, N transformation rates and total ^{15}N recovery. r_s = correlation coefficient, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. SoilM = soil moisture at a depth of 5 cm, soilT = soil temperature at a depth of 5 cm, Ndep = N deposition (kg N ha^{-1} yr^{-1}), CNff = C/N ratio of forest floor (L + FH), CNms = C/N ratio of mineral soil, pHms = pH-KCl of mineral soil.

environmental and soil physicochemical variables as can be seen in Fig. 4.2c. Correlations between the soil microbial community structure, N transformation rates and total ^{15}N recovery can be seen in Fig. 4.2d. Total recovery of $^{15}NH_4^+$ was negatively correlated to total AS concentration (r_s = -0.88, $p < 0.05$, n = 6) and gross nitrification rates (r_s = -0.94, $p < 0.05$, n = 6), while total recovery of $^{15}NO_3^-$ was not related to the microbial community nor to mineralization or nitrification rates.

4.4. Discussion

Significant differences in N cycling were observed between the edges and the interiors of the forest stands in our study. Gross mineralization rates were higher at the forest edges compared to the forest interiors in all forest types. Based on the $^{15}N_{rec}$ values, litter at the edge retained less N than the interior, while the FH layer and mineral soil captured more of the added inorganic N at the forest edge. Furthermore, differences in N cycling were associated with changes in the soil microbial community structure along the edge-to-interior transects.

4.4.1. Edge effects on microbial community structure and N cycling

The oak and pine stands hosted a larger microbial community at the forest edges than in the interior based on the PLFA and AS concentrations. The total living bacterial biomass (and more specifically of Gram+ bacteria) was higher at the forest edges, which received a higher atmospheric N load, and were drier and warmer than the forest interiors as measured by the soil moisture and temperature sensors (water filled pore space (WFPS) and soil temperature of 18 % and 11.5 °C at the edge of Qr2, WFPS of 27 % and 10.6 °C in the interior of Qr2, WFPS of 10 % and 12.1 °C at the edge of Pn2 and WFPS of 19 % and 11.3 °C in the interior of Pn2 averaged over 2 years), confirming our first hypothesis of bacterial dominance at forest edges. In general, most desiccation tolerant bacteria tend to be Gram+ due to their thicker cell wall (Schimel et al., 2007), which may help explaining their higher abundance at the forest edges. Nilsson et al. (2005), Demoling et al. (2008) and Kjøller et al. (2012) found that fungal biomass was negatively affected by N deposition. Also Zechmeister-Boltenstern et al. (2011) investigated the impact of N deposition on the soil microbial community in European forests and found that, N deposition was highly correlated with the ratio of fungi to bacteria, where sites with the highest N deposition were depleted in fungal PLFA. We could confirm these findings as the ratio of fungal to bacterial PLFA concentrations was negatively correlated to atmospheric N deposition. Högberg et al. (2013)

stated that bacteria, which commonly have lower C/N ratio than fungi, will be favoured over the ectomycorrhizal fungi in N-rich ecosystems, explaining bacterial dominance at high N deposition. The higher bacterial biomass might also be linked to the higher abundance of arthropod detritivores in these forest edges (De Smedt et al., 2016), increasing the accessible surface area of dead organic material for the microbial community (Harper et al., 2005). Furthermore, in our study, PLFA concentrations increased with an increasing pH of the mineral soil. Bacteria and fungi each have their optimum pH range, where bacteria often have a narrower pH range than fungi (Rousk et al., 2010). Malmivaara-Lämsä et al. (2008) also found that PLFA concentrations were positively related to soil pH in a study on edge effects in urban forest fragments. With a decreasing soil pH, the availability of biologically toxic aluminium increases, which affects the microbial community structure and the microbial activity (Bååth and Anderson, 2003). The upper mineral soil layers of the oak and pine forests were characterized by higher pH values at the edge than in the forest interior (Wuyts et al., 2013), favouring microbial presence at the forest edges.

Mineralization rates were higher at the forest edge compared to the forest interior, as stated in our second hypothesis. Staelens et al. (2012a) measured N transformation rates via a ^{15}N tracing model in an oak and a pine forest in northern Belgium. They obtained a gross mineralization rate of 1.1 and 0.4 mg N kg^{-1} day^{-1} and a gross nitrification rate of 0.28 and 0.6 mg N kg^{-1} day^{-1}, respectively for the oak and pine forest. Their N transformation rates for the oak forest are comparable to our values at the oak forest edge, while their N transformation rates of the pine forest are compatible to the values of the pine forest interior. The immobilization, net mineralization and net nitrification rates were characterized by large uncertainties. Our set-up was probably not adequate in covering the large spatial variation in inorganic N pools to obtain a reliable estimate of these rates. Therefore, we focused the discussion on the gross mineralization and nitrification rates. Högberg et al. (2013) suggested that fungi have a high immobilisation capacity and are less important as mediators of N mineralization as, in their study, mineralization was low when the fungi/bacteria ratio and soil C/N ratios were high. Their findings are consistent with our observations of higher gross mineralization rates and a higher abundance of Gram+ bacteria at the forest edges compared to the interior. Where mineralization is performed by generalists, autotrophic nitrification is attributed to a specific set of bacteria and Archaea (Wessén et al., 2011) and heterotrophic nitrification may be carried out by a wide range of microorganisms including fungi (Zhang et al., 2011). Nitrifying bacteria (genera of *Nitrosomonas* and *Nitrobacter*) are Gram- bacteria (Withers et al., 2001). There was no edge

effect on nitrification, contradictory to our expectations, which could be a reflection of the lack of an edge effect on Gram- bacteria. As mineralization was increased at the forest edges, but nitrification was not, this might be a possible bottleneck in the N cycle, preventing increased N leaching at the forest edge. Nitrate can be transformed to N_2 via denitrification if enough water accumulates in the subsoil (B horizon, see Fig. A-I) and sufficient organic C is available. However, we did not encounter water stagnation when sampling mineral soil of these well-drained sandy Podzols.

Nadelhoffer et al. (2004) and Wessel et al. (2013) found that forest floor and soil remained the dominant sinks of N even seven years after ^{15}N addition, with highest tracer recoveries in organic soil, followed by mineral soil (0 - 20 cm), in temperate oak stands at Harvard Forest (USA). We focused on the soil (organic and mineral layers), since we wished to monitor the movement of external ^{15}N entering the forest soil via throughfall. In the sprayed plots, no trees or understorey vegetation were present. However, roots were present in the injected mineral soil and could have contributed to uncontrolled uptake and removal of ^{15}N from the soil. Recovery of $^{15}NH_4^+$ in litter was higher in the interior, but recovery of $^{15}NO_3^-$ was higher in the FH and mineral soil layers at the oak forest edge. Templer et al. (2012) suggested that N recovery can be used as a proxy of ecosystem N retention. We hypothesized that higher nitrification rates would be expected to lead to higher N losses and thus lower ^{15}N recovery in the forest edge. However, as Wuyts et al. (2011) measured lower inorganic N leaching in the first 10 to 20 m of the forest edge, $^{15}N_{rec}$ percentages could also be higher at the forest edge. Indeed, in the oak stands, recovery of $^{15}NO_3^-$ was higher at the edge than in the forest interior, providing a buffer for additional N input. The higher recovery of $^{15}NO_3^-$ at the oak forest edge was consistent with the higher N stocks in the mineral soil layer found by Remy et al. (2016b, see Chapter 2) and the lower leaching losses observed by Wuyts et al. (2011) at the forest edge compared to the interior. The pine and spruce stands showed $^{15}N_{rec}$ values above 100 %. Recoveries of > 100 % could be due to sampling and analytical errors (Dail et al., 2001) or overlap of ecosystem pools (Templer et al., 2012). The latter was not the case in our study, since we sampled four distinct soil layers (L, FH and mineral soil from 0 – 10 and from 10 – 20 cm deep). In the pine stand, both $^{15}NH_4^+$ and $^{15}NO_3^-$ were stored in the litter layer of the forest interior, while $^{15}NH_4^+$ was retained in the FH layer at the forest edge. In the spruce stand, more $^{15}NO_3^-$ reached the FH layer at the edge and $^{15}NH_4^+$ was stored in the mineral soil at the edge (although not significant). Remy et al. (2016b) suggested that lower N stocks in the forest floor were linked to faster litter degradation at the edge due to microclimatic gradients and a different microbial and invertebrate abundance and community at the edge (De Smedt et al., 2016), hereby

transferring nutrients to deeper soil layers (see Chapter 2, § 2.4.1.). Transportation towards soil layers below the sampled soil depth and uptake of ^{15}N by other forest compartments (e.g. roots, trees) could also have contributed to the observed $^{15}N_{rec}$ values. However, Staelens et al. (2012a) found that $^{15}N_{rec}$ in the roots contributed < 2 % to the total $^{15}N_{rec}$ of the sampled soil-root system. N retention mechanisms might be abiotic as correlation analyses per soil layer (FH and MS) confirmed the lack of a link between $^{15}N_{rec}$ and PLFA concentrations (data not shown). Inorganic N in soil might be chemically or physically protected from microorganisms via one of the following pathways: selective preservation due to recalcitrance, spatial inaccessibility of decomposer organisms due to occlusion, intercalation, hydrophobicity and encapsulation, or stabilization by interaction with mineral surfaces and metal ions (Lützow et al., 2006). However, the sampled mineral soil of these sandy Podzols is characterized by low amounts of clay minerals. Therefore, the most likely stabilization mechanisms are biochemical stabilisation via the formation of recalcitrant phenolic compounds and microbial immobilisation.

4.4.2. Forest type effects on microbial community structure and N cycling

In the oak forests, microbial biomass decreased strongly with distance to the edge, while microbial biomass decreased slightly with distance to the edge in the pine stands, and stayed rather constant in the spruce stand. Phospholipid fatty acid (total, fungal, bacterial and ratio of fungal to bacterial PLFA) and total AS concentrations were negatively correlated to the C/N ratio of the forest floor (L and FH layer). Deciduous oak forests are characterized by lower C/N ratios than evergreen pine and spruce forests, due to the lower lignin and higher N content in oak leaves than in needles (Cools et al., 2014). Increased N deposition lowers C/N ratio, stimulating microbial growth and especially bacterial growth as fungi have higher C/N ratios than bacteria and therefore are expected to have lower N demands (Strickland and Rousk, 2010). Griepentrog et al. (2014) showed that a low C/N ratio and high N availability retards the decomposition of AS residues in soil, as microorganisms are no longer forced to use organic N sources when N limitation ceases. The link between the C/N ratio and PLFA might also be indirect as the C/N ratio reflects the quality of the soil organic matter. Consequently, organic matter with a high C/N ratio will favour the occurrence of fungi.

Furthermore, the PLFA concentration of Gram- bacteria was highest in the oak stands, coinciding with the highest nitrification rate of the three forest types. Gross nitrification was positively correlated with total AS concentration, as dead microbial biomass may provide a

substrate for nitrification and living microbial biomass might contain nitrifying bacteria and fungi. Gross nitrification rates were negatively correlated to the recovery of $^{15}NH_4^+$, as nitrification converts NH_4^+ to NO_3^-, hereby decreasing NH_4^+ concentrations in soil. Nitrification was also negatively correlated with the C/N ratio of the forest floor (L and FH). Christenson et al. (2009) found that gross mineralization and nitrification rates were negatively related to C/N ratio in deciduous forests in north-eastern USA, which was confirmed in our study for the nitrification rates. The higher nitrification rate in the oak stand, compared to the pine and spruce stands, will presumably lead to a higher availability of NO_3^-. Indeed, in a previous study, Wuyts et al. (2011) have shown that the oak stand (Qr2) lost high amounts of N via leaching, i.e. 35 and 20 kg N ha^{-1} yr^{-1} in the edge and in the interior.

Staelens et al. (2012a) hypothesized that the microbial community could explain the differences in mineralization and nitrification rates, found in adjacent oak and pine stands as C/N ratio, soil type, stand history, tree age and soil temperature were similar. Bengtsson et al. (2003) also found that differences in gross N mineralization rates in three deciduous forests were strongly related to the microbial community. Buurman et al. (2007) observed mottling in all horizons of some well-drained Podzols on quartz sands in the Netherlands, Belgium and Germany due to the selective removal of organic matter. Phospholipid analysis suggested that the removal of organic matter was due to a combination of bacteria, fungi, and actinomycetes. However, in our study sites we did not observe this white mottling within the sampled soil.

In our study, mineralization rates were also positively correlated to soil temperature, as the oak and the pine stands were characterized by higher soil temperatures than the spruce stand (averaged soil temperature of 11.3 °C in the oak and pine stands and 8.6 °C in the spruce stand over 2 years). Temperature is recognized as a key factor regulating many terrestrial biogeochemical processes, such as soil respiration and mineralization (Rustad et al., 2001). Guntiñas et al. (2012) showed that nitrogen mineralization increased with soil temperature, where the sensitivity to temperature was maximal at 25 °C.

Dise et al. (2009) found that N retention varied between forests in Europe, with high N retention at low atmospheric N deposition rates (< 8 kg N ha^{-1} yr^{-1}) and lower retention at higher N deposition. Templer at al. (2012) showed that N retention decreased from > 90 % at sites receiving < 7 kg N ha^{-1} yr^{-1} to < 60 % at sites receiving > 11 kg N ha^{-1} yr^{-1}. Moreover, above a fertilization rate of 46 kg N ha^{-1} yr^{-1}, N addition decreased ^{15}N retention. The oak

forest edge received less atmospheric N deposition than the edge of the pine stand and was characterized by stronger N retention at the edge. The pine forest edge was subjected to higher N deposition values than the threshold value of 46 kg N ha^{-1} yr^{-1}, set by Templer et al. (2012) and the litter layer lost more N at the forest edge compared to the interior, but retention of N still occurred in the FH layer, where N is probably immobilised via abiotic pathways.

4.5. Conclusion

Biomass of Gram+ bacteria was higher at the forest edges compared to the forest interiors and was associated to higher N mineralization rates. There was no significant impact of edge proximity on Gram- bacteria, which was reflected in the lack of an edge effect on the nitrification rates. Furthermore, forest type additionally affected microbial N cycling through differences in atmospheric N deposition, and quality and quantity of the released organic material. Gross and net nitrification rates differed between the forest types, where the oak stands were characterized by higher nitrification rates than the pine and spruce stands. Despite the high mineralisation and nitrification rates, the oak stand retained N in the FH layer and mineral soil at the edge. In all forest types, the forest interior retained more N in the litter layer, while N was stored in deeper soil layers at the edge. Our results contribute to elucidating the changes in N cycling at forest edges, as they are compatible with previous research at the same forest stands, showing higher arthropod abundance, higher mineral soil N stocks and lower leaching losses at forest edges. Overall, our results indicated that the specific characteristics of the forest edge (atmospheric deposition, microclimate and soil physicochemical characteristics) increased microbial biomass, N turnover (gross mineralization) and storage capacity beneath the litter layer and changed the microbial community structure. Forest edges are a dominant feature in many landscapes of Western Europe. Hence, more research should be conducted to validate our observations for other forest and soil types. Moreover, it is unclear how long these forest edges will be able to store additional N beneath the litter layer under ongoing high atmospheric deposition.

5. Edge effect on litter decomposition and nutrient release

After: Remy E., Wuyts K., Van Nevel L., De Smedt P., Boeckx P., Verheyen K.. Driving factors behind litter decomposition and nutrient release at temperate forest edges (submitted).

Abstract

Forest edges have become important features in landscapes worldwide. Edges are exposed to a different microclimate and higher atmospheric nitrogen (N) deposition compared to forest interiors. It is, however, unclear how microclimate and elevated N deposition affect nutrient cycling at forest edges. We studied litter decomposition and release of N, phosphorus (P), exchangeable cations (EC), and C/N ratios during 18 months via the litterbag technique along edge-to-interior transects in two oak (*Quercus robur* L.) and two pine (*Pinus nigra* ssp. *laricio* Maire and ssp. *nigra* Arnold) stands in Belgium. Secondly, litter from edge and interior was interchanged to test the impact of litter position and litter quality. Thirdly, litter from the interior was placed in Open Top Chambers, to scrutinize the role of edge soil fauna on litter decomposition. Increased litter mass loss and nutrient release were observed at the edge compared to the interior in the oak stands and were governed by soil acidity and forest floor C/N ratio. In the pine stands, N and P release was higher at the edge compared to the interior. The contribution of each driving factor (litter position, litter quality and edge soil fauna) depended on the specific characteristics of the forest edge. We demonstrated an edge effect on litter decomposition and nutrient release, caused by an interplay of the edge microclimate, atmospheric deposition, soil characteristics, litter quality and soil fauna. Consequently, edge effects must be accounted for when quantifying ecosystem processes, such as litter decomposition and nutrient cycling in fragmented landscapes.

5.1. Introduction

Central and Western European landscapes are characterized by small forest remnants resulting from a long history of land-use change (Hofmeister et al., 2013). Consequently, forest edges have become important features in the landscape (Decocq et al., 2016). These edges differ substantially from forest interior zones in regard of, amongst other factors, microclimate, atmospheric deposition, physicochemical soil conditions, nutrient and carbon (C) stocks and fluxes, forest structure and faunal and floral species composition and dynamics (e.g., Matlack 1993; Chen et al., 1995; De Schrijver et al., 2007; Wuyts et al., 2008a, 2008b; Remy et al., 2016a, 2016b, see Chapters 2 and 3). Decomposition of leaf litter is a major source of nutrients in forest ecosystems and influences the proportion and persistence of nutrient and C retention in soil (Cotrufo et al., 2013). However, the effects of forest fragmentation on litter decomposition in temperate forests are poorly understood (Herbst et al., 2007).

The rate of litter decomposition is affected by soil temperature, soil moisture, soil chemical conditions, the composition of the decomposer community, and the litter quality (Sariyildiz, 2008). Due to increased solar radiation, wind and higher evapotranspiration rates, forest edges generally have higher soil temperatures and a lower soil and litter moisture content than interiors (Herbst et al., 2007; Riutta et al., 2012). Ritter et al. (2005) observed considerable microsite variation in soil moisture content of forest edge versus interior. In temperate regions, moisture content is often a stronger regulator of litter decomposition than temperature (Aerts, 2006). Decomposers also play a critical role in nutrient cycling in forests, as they drive the soil C cycle by mineralizing organic matter for their growth (Manzoni et al., 2012; David, 2014). Malmivaara-Lämsä et al. (2008) found decreased microbial biomass and activity at forest edges (i.e. a decrease of 30 to 45 % up to 20 m from the forest edge) and implied that this would lead to decreased litter decomposition rates and consequently to altered nutrient cycling. In the majority of terrestrial ecosystems, earthworms represent the largest soil fauna biomass (Lavelle and Spain, 2001). Zeithaml et al. (2009) found higher earthworm density and biomass at the forest edge in oak and mixed oak-pine forests in the Czech Republic. However, in temperate forests growing on acid sandy soils, the most abundant macrofauna groups are woodlice (Isopoda) and millipedes (Diplopoda) (David and Handa, 2010), which are sensitive to changes in temperature and moisture (Edwards et al., 2010). De Smedt et al. (2016) showed that woodlice abundance decreased exponentially from the forest edge towards the forest interior, while millipede abundance showed an

optimum after the first 10 m from the forest edge, between edge and interior. They attributed these trends to the specific desiccation tolerance of the different species.

Litter chemistry and stoichiometry with regard to C, nitrogen (N) and phosphorous (P) is an important regulator of litter decomposition, as higher litter C/N and C/P ratios negatively affect N and P mineralization, respectively (Mooshammer et al., 2012). Cools et al. (2014) showed that the C/N ratio of the organic layer (litter and fragmented litter and humus) and mineral topsoil mainly depends on tree species, where deciduous tree species are characterized by lower C/N ratios than evergreen tree species. Moreover, Spangenberg and Kölling (2004) and Wuyts et al. (2011) measured lower C/N ratios of the organic layer at the edge, which was exposed to higher N deposition levels than the forest interior. Furthermore, the litter concentration of exchangeable cations, such as Ca^{2+}, also affects decomposition rates (e.g. Hobbie et al., 2006).

The sparse studies on the effects of forest fragmentation on decomposition mainly report on observations in (sub)tropical forests (Didham, 1998; Vasconcelos and Laurance 2005; Moreno et al., 2014;) or on wood decomposition (González et al., 2008; Crockatt and Bebber, 2015). Experimental studies that reveal the driving factors of litter decomposition in temperate forest edges are lacking. Up to now, only Riutta et al. (2012) investigated edge effects on litter decomposition rates in temperate deciduous forests and experimentally tested the moisture limitation hypothesis. They observed lower decomposition rates in the edge compared to the interior, which was attributed to moisture limitation at the drier forest edge.

We wanted to further elucidate the knowledge gap on litter decomposition and nutrient cycling in the highly fragmented landscapes of Western Europe. Therefore, we designed a unique tripartite experimental set-up to determine edge influence on leaf litter decomposition in temperate forests and elucidate the role of litter quality, litter position and macrofauna therein. The study was performed in two oak and two pine stands, situated in an agricultural landscape in northern Belgium. Firstly, edge effects on litter decomposition and nutrient release were assessed during 18 months along edge-to-interior transects using the litterbag technique. Secondly, litter of edge and interior (128 m) was interchanged to determine the importance of edge conditions (microclimate, atmospheric deposition, soil decomposer community and soil physicochemical conditions) and litter quality on the decomposition rate. Thirdly, litter of the forest interior was placed in Open Top Chambers (OTC), which simulated

edge microclimate (warmer) in the forest interior in absence of the specific forest edge community of litter and soil-dwelling fauna. We hypothesized that (i) due to the higher N deposition and contrasting microclimate at the edge, initial leaf/needle litter decomposition and nutrient release would be faster at the forest edge than in the interior, (ii) the edge conditions would stimulate leaf/needle litter decomposition, irrespective of the litter quality, (iii) edge leaf/needle litter would break down faster than interior litter, irrespective of its location (edge/interior), due to the improved litter quality (lower C/N ratio, more EC) of edge litter compared to interior litter, and (iv) due to the absence of the specific edge decomposer macrofauna, leaf/needle litter decomposition of interior litter in the OTC would be slowed down compared to interior litter at the forest edge.

5.2. Material and methods

5.2.1. Study area

Four forest edges were selected for detailed characterization, comprising tree species relevant for their respective region. The forests are situated in Belgium and comprise of a pedunculate oak (*Quercus robur* L.) stand in Wortegem, West Flanders (Qr1), a second pedunculate oak stand in Ravels, Antwerp (Qr2), an Austrian pine (*Pinus nigra* ssp. *nigra* Arnold) stand in Zedelgem, West Flanders (Pn1), and a Corsican pine (*P. nigra* ssp. *laricio* Maire) stand in Ravels, Antwerp (Pn2). All stands are even-aged monocultures and grow on acid, sandy quartz-dominated Podzols. Previous land use was in all cases heathland until afforestation in last century. The considered forest edges are facing the locally prevailing wind direction (southwest), which creates the steepest edge gradients in throughfall deposition (Draaijers et al., 1988). An overview of the stand and physicochemical characteristics can be found in Table 1.1. Mean annual air temperature is 10.5°C and mean annual precipitation is 800 mm in Belgium (data obtained from the nearest weather station operated by the Royal Meteorological Institute of Belgium, 1981-2010). The understory vegetation is composed of ferns (*Dryopteris dilatata* and *Dryopteris carthusiana*) and grasses (*Molinea caerulea* and *Holcus* sp.) in the pine stands, with brambles (*Rubus fruticosus* agg.) in Pn1. The understory vegetation in the edges of the oak stands is characterized by brambles (*Rubus fruticosus* agg.).

5.2.2. Experimental set-up

Decomposition of leaf and needle litter was studied via the litterbag technique. In a first experiment (litterbag series A, Fig. 5.1), litter decomposition was followed over time along

the edge-to-interior transects. Litterbags were placed at the edge (0 m), at 16 m, at 64 m and at 128 m from the edge to detect differences in the rate of litter decomposition in function of edge proximity. In a second experiment, fresh litter of the forest interior and forest edge were interchanged to test the impact of the edge conditions (microclimate, atmospheric deposition, soil decomposer community and soil physicochemical conditions) and litter quality (litterbag series B, Fig. 5.1) on litter decomposition. In a third experiment (litterbag series C, Fig. 5.1), litter of the forest interior was placed in the forest interior in Open Top Chambers (OTC), which creates an edge microclimate (warmer than in the forest interior, De Frenne et al., 2010) in the absence of the edge decomposer macrofauna. Soil moisture was not significantly affected by the OTCs (De Frenne et al., 2010), nor the arthropod community as they were able to crawl under the OTCs (pers.comm.).The litterbags were 20 by 20 cm in size and consisted of a wire mesh (5 x 5 mm), fitted with a nylon mesh (mesh size of 1 x 1 mm) at the bottom, which makes contact with the soil. In this way, litter loss was prevented, but horizontal entry of most soil fauna was still allowed. In total, 420 litterbags were manufactured by hand. In all stands, falling leaves and needles were intercepted during winter 2013 on nets placed at the four distances (0, 16, 64 and 128 m). The intercepted leaves and needles were air-dried during 7 days. Litterbags were filled with 10 g of air-dried leaves or needles. In the pine forest Pn2, very low amounts of litter had fallen at the forest edge. Hence, the B series in this forest contained only 5 g of litter. Litterbags were anchored into the litter layer of the forest floor by means of a bended iron wire coated with a layer of synthetic material. All experiments were conducted from November 2014 until June 2016.

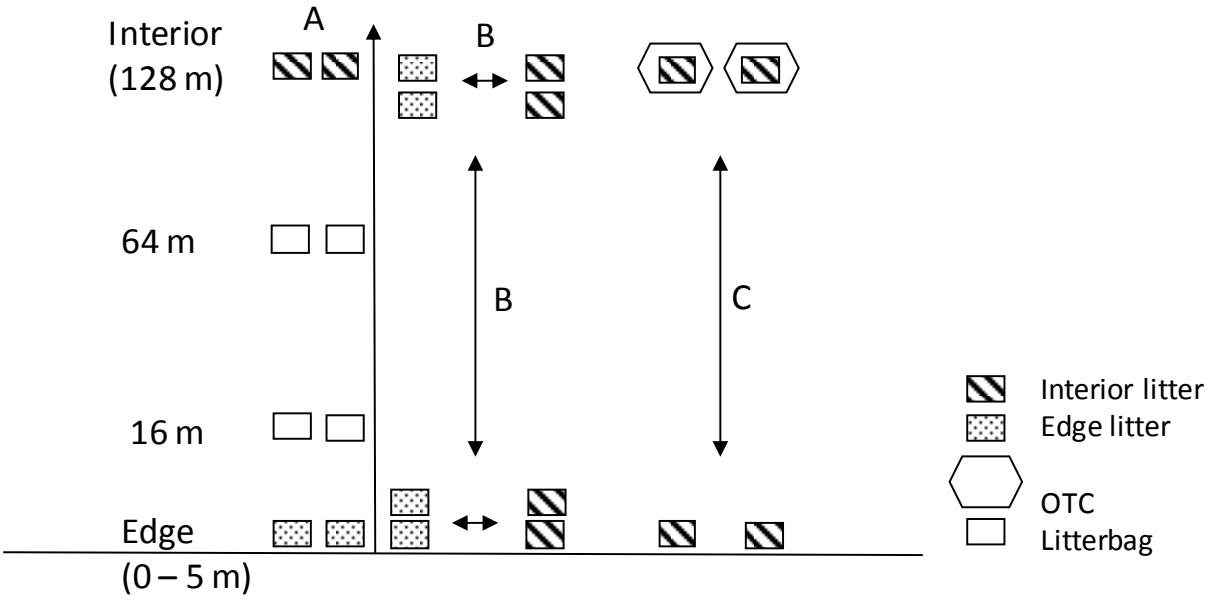

Fig. 5.1: Schematic overview of the set-up of litterbag series A, B and C.

5.2.3. Sampling and chemical analysis

Two litterbags were collected from each distance and from each series (A, B and C) every 3 months (after 3, 6, 9, 12, 15 and 18 months). Retrieved litter samples were dried at 25°C for 2 days and weighed. Hereafter, they were dried at 65 °C for 2 days, analyzed for oven-dry mass and milled (ZM1, Retsch, Germany). In this way, the correction for the difference in mass loss between air-dried litter and oven-dried litter was known (Bärlocher, 2005). Nitrogen and carbon (C) concentrations were measured by a CNS elemental analyzer (vario Macro Cube, Elementar, Germany). Leaf and needle samples were destructed (by addition of 2 ml HNO_3 and 0.4 ml of $HClO_4$ to 75 mg of sample in Teflon pots, followed by a dilution up to 50 ml) prior to phosphorus (P), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) concentration measurements. P concentration was measured colorimetrically on 5 ml of destructed sample (Cary 50 Spectrophotometer, Agilent Technologies, USA) by addition of 2 ml of a mixture of H_2SO_4, NH_4 para-molybdate, polyvinyl alcohol and malachite green. K^+, Mg^{2+} and Ca^{2+} concentrations were measured on the remaining destructed sample by atomic absorption spectrometry (SpectrAA 240FS, Agilent Technologies, USA) after a 10 % addition of CsCl modifier (25 mg CsCl in 50 ml HCl 37 %, diluted to 1 l). Lignin concentrations were measured at the Institute for Agriculture and Fisheries Research (ILVO), according to the methods of Van Soest et al. (1991) on a bulk sample of air-dried litter of each litter type at each distance.

5.2.4. Statistical analyses

When strong curvatures are observed in the decomposition dynamics, the single exponential curve, developed in detail by Olson (1963, Eq. 5.1) can be less appropriate

$$x_t/x_0 = e^{-kt} \qquad \text{(Eq. 5.1)}$$

where x_t is the remaining amount of litter at time t (g), x_0 is the initial amount of litter (g), *t* is the time (yr) and *k* is the decomposition rate (yr^{-1}). Therefore, we used the conceptual approach of Rovira and Rovira (2010) comparing three possible patterns in which the decomposition rate varies with time: (1) exponential rate decrease, (2) wave-form dynamics, simulating seasonal rhythms, and (3) rational-type dynamics, involving a rate increase in the initial phase, followed by a gradual decrease. Next to these equations, our dataset was also fitted to Olson's single exponential model (Eq. 5.1), to ensure that the considered equations improved the fit. The most appropriate model was chosen based on the adjusted coefficient

of determination (Eq. 5.2) and the corrected Akaike Information Criterion (AIC_c) value (Eq. 5.3 and Eq. 5.4)

$$adjusted\ R^2 = 1 - \frac{(1-R^2)\,(n-1)}{n-K-1} \quad \text{(Eq. 5.2)}$$

where R^2 = 1 – residual sum of squares/corrected sum of squares, n is the sample size and K is the number of parameters involved in the model.

$$AIC = n\,log(\sigma^2) + 2K \quad \text{(Eq. 5.3)}$$

$$AIC_c = AIC + \frac{2K\,(K+1)}{n-K-1} \quad \text{(Eq. 5.4)}$$

where AIC_c is the corrected AIC value for a small dataset (low n values) and σ^2 is the residual variance. The adjusted R^2 values did not differ much between Olson's single exponential equation and the equations described by Rovira and Rovira (2010). Moreover, if any of the proposed equations by Rovira and Rovira (2010) is preferable over the classic Olson's model, then $AIC_{Olson} > AIC_{Eq}$. Consequently, the difference in AIC values ($\Delta AIC = AIC_{Eq} - AIC_{Olson}$) would be negative. As both ΔAIC and ΔAIC_c were positive, the replacement of Olson's model by any of the proposed equations was not justified, in spite of the possible increase in the adjusted R^2. The values of the decomposition rate *k* are presented in Table 5.1.The relative remaining litter mass in the litterbags is presented in Fig. 5.2.

Significant differences in initial litter concentrations and the decomposition parameter along the edge-to-interior transects were assessed via one-way ANOVA for each forest type (Table 5.3). Pearson correlation coefficients were calculated between initial litter concentrations (N_0, C_0, mass based C/N_0 ratio, $lignin_0$, P_0 and EC_0, Table 5.2), relative litter mass loss and the decomposition parameter (*k)* of series A for each forest type to explore which litter characteristics influence litter decomposition dynamics (Table 5.4).

Absolute masses of the elements in the litterbags (mg) were calculated by multiplying element concentrations with the remaining dry mass at each collection date, enabling us to make statements on nutrient release (Fig. 5.3). Concentrations of K^+, Mg^{2+} and Ca^{2+} (mg kg^{-1}) were converted to amounts in milliequivalents (meq) and their sum is further referred to as exchangeable cations (EC). Nutrient releases, i.e. differences between remaining nutrient masses after 18 months and the initial mass were calculated absolutely (in mg or meq) and relatively (in %, Table 5.5).

Next, a two-way analysis of variance (ANOVA) was performed on the mass loss and nutrient release data of series A of each stand to assess the influence of the discrete predictor variables distance to the forest edge (4 levels: 0, 16, 64 and 128 m), time of sampling (6 levels: 3, 6, 9, 12, 15 and 18 months) and their interaction on relative mass loss, nutrient release of N, P and EC and relative changes in C/N ratios. A Tukey's HSD post-hoc test was performed to explore differences between the distances along the edge-to-interior transects (0, 16, 64, 128 m), at a significance level of $p < 0.05$ (Table 5.6).

To assess the role of litter position (microclimate, atmospheric deposition, decomposer community and soil physicochemical conditions inherent to the edge and interior) and litter quality (edge, interior) in decomposition edge effects, two-way ANOVA's were performed on data of mass loss (in %), nutrient release (in %) and relative changes in C/N ratios of series A, B and C after 18 months for each stand. A Tukey's HSD post-hoc test was performed to explore differences between the litter positions (edge, interior, OTC), with a significance level of $p < 0.05$.

Correlations (Pearson correlation coefficients) were assessed between relative changes in litter mass and nutrient amounts on the one hand, and soil physicochemical and environmental characteristics (pH of mineral soil, C/N ratio of forest floor and mineral soil, atmospheric N deposition, soil temperature and moisture (previously determined by Wuyts et al. (2009, 2013) and Remy et al. (2016) along the same edge-to-interior transects) on the other hand, for each forest type. Model fitting was performed in SPSS Statistics 24 for Windows, while the other statistical analyses were performed in R 3.3.1 (R Development Core Team, 2016).

5.3. Results

5.3.1. Mass loss and nutrient release along edge-to-interior transects

Mass loss

The relative remaining mass in the litterbags, fit with Olson's single exponential model (Eq. 5.1), decreased over time (Fig. 5.2). This decrease in mass loss was more pronounced in the oak stands compared to the pine stands, where litter mass loss was more or less constant over a time period of 18 months. In the oak stands, litter mass at 16 m, 64 m and 128 m stayed rather constant at first and decreased only after 12 months, while litter mass at the edge decreased continuously. The mean decomposition rate *k* ranged between 0.3

and 1.0 yr^{-1} and was highest at the edge of the oak stand Qr1 (Table 5.1), coinciding with the lowest relative remaining mass (Fig. 5.2a). A high mean decomposition rate will lead to a low mean residence time (MRT), as *k* and MRT are inversely related. However, as decomposition of the recalcitrant oak and pine litter was not finished after 18 months, the MRT has no significant biological meaning yet. In the oak stands, Tukey HSD post-hoc tests revealed that relative remaining litter mass at the edge was significantly lower than litter mass at 16 m, 64 m and 128 m over the whole period of 18 months (Table 5.6). The remaining litter mass after 18 months was 7.3 ± 1.2 % and 28.5 ± 2.1 % of initial mass, respectively at the edge of Qr1 and Qr2, and 56.2 ± 4.2 % and 45.7 ± 5.6 % in the forest interior (128 m) of, respectively, Qr1 and Qr2 (Fig. 5.2a and 5.2b). In the pine stands, litter mass loss was rather constant along the edge-to-interior transects (Table 5.6). Remaining litter mass after 18 months was 47.3 ± 9.6 % and 33.6 ± 12.2 %, respectively at the edge of Pn1 and Pn2 (Fig. 5.2c and 5.2d) and 40.5 ± 4.5 % and 47.3 ± 2.2 % of the initial mass in the interior (128 m) of Pn1 and Pn2, respectively.

Table 5.1: Decomposition parameter *k* (average ± standard deviations) obtained with Olson's single exponential model (Eq. 5.1, n = 12) applied to litter mass data of series A and adjusted R^2 of the fits.

	Oak			
	Qr1		Qr2	
Distance (m)	k (yr^{-1})	R^2	k (yr^{-1})	R^2
Edge (0 - 5)	1.009 ± 0.550	0.72	0.683 ± 0.145	0.81
16	0.556 ± 0.337	0.56	0.354 ± 0.252	0.21
64	0.471 ± 0.216	0.60	0.418 ± 0.178	0.71
Interior (128)	0.402 ± 0.125	0.65	0.425 ± 0.158	0.80
	Pine			
	Pn1		Pn2	
Edge (0 - 5)	0.479 ± 0.203	0.76	0.455 ± 0.169	0.83
16	0.591 ± 0.387	0.61	0.391 ± 0.146	0.89
64	0.542 ± 0.333	0.60	0.453 ± 0.241	0.82
Interior (128)	0.644 ± 0.225	0.77	0.438 ± 0.126	0.92

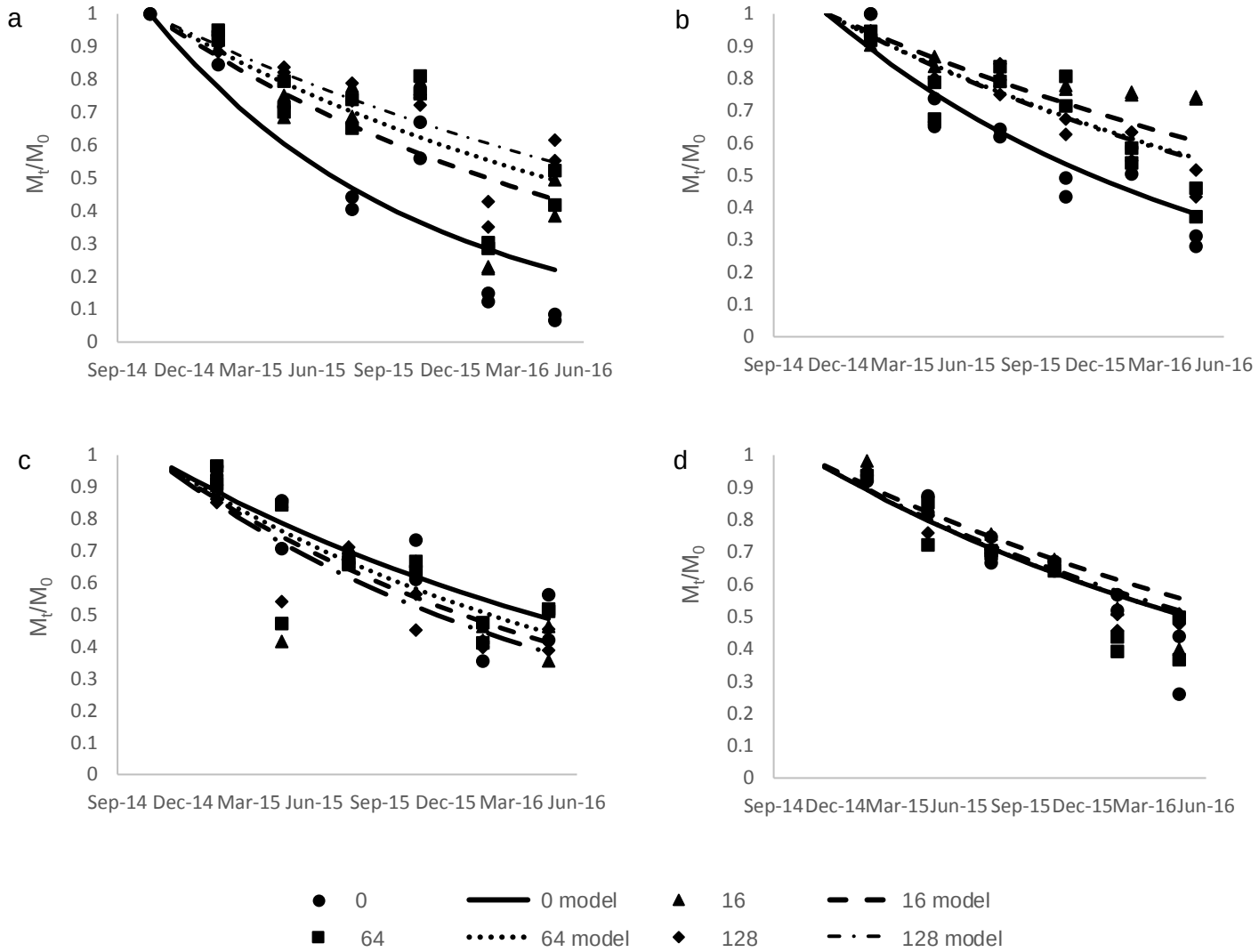

Fig. 5.2: Relative remaining mass (relative to initial mass, M_t/M_0) in the litterbags of series A during 18 months of litter decomposition and modelled data according to Olson's single exponential model (Eq. 5.1) for the four distances (0, 16, 64, 128 m) along the edge-to-interior transects in the oak a) Qr1 and b) Qr2 and pine stands c) Pn1 and d) Pn2; see decomposition parameter *k* in Table 5.1.

Nutrient release

Initial concentrations of N, C, lignin to N, P and exchangeable cations (EC) and initial C/N ratios in intercepted litter are presented in Table 5.2. Only the initial concentrations of EC of pine litter decreased significantly with distance to the edge (Table 5.3). Lignin to N ratios were significantly lower ($t = -4.32$, $p = 0.001$, $n = 8$) in the oak stands compared to the pine stands, while EC amounts were significantly higher ($t = 6.43$, $p < 0.001$, $n = 8$) in the oak stands compared to the pine stands.

Table 5.2: Initial litter concentrations of nitrogen (N_0), carbon (C_0), lignin to N ratio (lignin/N_0), phosphorus (P_0) and exchangeable cations (EC_0), and initial C/N ratios (C/N_0) intercepted at the four distances along the edge-to-interior transects in the oak (Qr1 and Qr2) and pine (Pn1 and Pn2) stands.

Species	stand	Distance (m)	N_0 (%)	C_0 (%)	lignin/N_0	P_0 (mg kg^{-1})	EC_0 (meq kg^{-1})	C/N_0
Oak	Qr1	0	1.3	49.9	19.6	825.1	792	37.0
	Qr1	16	1.3	49.7	23.5	696.1	799	37.1
	Qr1	64	1.7	50.5	16.3	878.3	636	29.1
	Qr1	128	1.3	51.6	22.4	752.5	572	38.4
	Qr2	0	1.7	50.5	16.4	493.1	646	31.5
	Qr2	16	1.6	51.5	20.2	489.6	519	31.5
	Qr2	64	1.5	51.4	22.2	656.3	532	33.4
	Qr2	128	1.4	50.5	22.5	728.4	502	35.7
Pine	Pn1	0	0.7	53.0	37.6	222.6	382	72.5
	Pn1	16	0.9	53.8	32.9	221.4	299	62.0
	Pn1	64	0.8	53.3	36.1	237.6	256	67.7
	Pn1	128	1.0	52.7	25.2	276.8	217	54.7
	Pn2	0	1.2	53.0	20.4	654.0	459	45.6
	Pn2	16	1.0	53.2	29.5	382.5	265	54.8
	Pn2	64	0.9	53.3	30.8	307.8	215	57.4
	Pn2	128	1.1	53.4	27.8	319.5	199	50.7

Table 5.3: P-values of the One-way ANOVA testing the effect of the predictor variable distance to the forest edge on the response variables initial litter concentrations (N_0, C_0, mass based C/N_0 ratio, P_0, $Lignin_0$, EC_0) and the decomposition parameter (*k*) for the oak and pine stands ($n = 8$), * $p < 0.05$.

	Oak	Pine
N_0	0.539	0.611
C_0	0.279	0.680
C/N_0	0.498	0.546
Lignin/N_0	0.311	0.627
P_0	0.359	0.486
EC_0	0.110	0.019*
k	0.127	0.437

Correlation analyses between initial litter characteristics (N_0, C_0, mass based C/N_0 ratio, P_0, $Lignin_0$, EC_0; see Table 5.2), and litter mass loss and the decomposition parameter *k* for oak and pine separately, revealed a significant positive correlation between the decomposition parameter *k* and the initial concentration of EC for the oak stands. In the pine stands, litter mass loss was negatively correlated to initial lignin concentration (Table 5.4).

Table 5.4: Pearson correlation coefficients between initial litter concentrations (N_0, C_0, mass based C/N_0 ratio, P_0, $Lignin_0$, EC_0), and absolute litter mass loss and the decomposition parameter (*k*) for the oak and pine stands (n = 8), * $p < 0.05$.

	Oak	
	k	Mass loss
N_0	-0.232	-0.191
C_0	-0.673	-0.663
C/N_0	0.195	0.153
$Lignin_0$	-0.311	-0.272
P_0	0.237	0.312
EC_0	0.764*	0.665
	Pine	
N_0	-0.229	0.608
C_0	-0.087	-0.085
C/N_0	0.261	-0.616
$Lignin_0$	0.075	-0.725*
P_0	-0.442	0.488
EC_0	-0.161	0.666

Absolute and relative nutrient releases, i.e. differences between remaining nutrient masses after 18 months and the initial mass can be found in Table 5.5. At the edge of the oak stands, N amounts remained constant until one year of decomposition and decreased after this sampling moment (November 2015, Fig. 5.3a). After 18 months of litter decomposition, N loss was significantly higher at the oak edges compared to the interiors (Table 5.6). Nitrogen loss at the edge was increased by 2 orders of magnitude (or 124 mg) and by 80 % (or 56 mg), respectively in Qr1 and Qr2. The significant interaction term showed that N loss fluctuated along the edge-to-interior transect over time in Qr2 (Table 5.6). In Pn1, N loss was 40 % (or 9.5 mg) lower at the edge compared to the interior (Table 5.6). At the edge of Pn2, the N amounts dropped in the first 3 months, stayed rather constant in the following 12 months and decreased again in the last 3 months of the experiment, whereas the interior (128 m) showed a slow continuous decrease in pine litter N amount (Fig. 5.3a). In Pn2, N loss of edge litter was twice as high as N loss of interior litter (or 33 mg, Table 5.6).

In the oak stands, litter C/N ratios decreased during the first 6 months and stayed constant hereafter, while pine litter C/N ratios showed the opposite pattern: a constant C/N ratio during the first 6 months and a slow decrease over the remaining course of the experiment over all distances (Fig. 5.3b). Litter C/N ratios were significantly higher at the edge of the pine stands (on average respectively 18 % for Pn1 and 15 % for Pn2) compared to the interior (Fig. 3b, $p < 0.05$ for Pn1 and Pn2, $n = 28$). Relative changes in litter C/N ratios were significantly affected by distance to the forest edge with higher relative changes in litter C/N ratios at the oak edge of Qr2 (40 %) and pine edge of Pn2 (40 %), but lower relative changes at the pine edge Pn1 compared to the interior (28 %, Table 5.6).

In the oak stands, the amounts of P decreased during the first 3 months at all distances, followed by an increase (but not at the edge of Qr1) during the following 9 months. After 12 months, P amounts decreased again at all distances in both stands (Fig. 5.3c). In the oak stands Qr1 and Qr2, there was a significant effect of distance to the forest edge on P loss (Table 5.6). P loss at the oak edges was increased by 72 % (or 4.9 mg) and 30 % (or 2.1 mg), respectively for Qr1 and Qr2. In the pine stands, P amounts stayed constant, except at the edge of Pn2, where a sharp decrease in P amount occurred in the first 3 months (Fig. 5.3c). In the pine stands, P loss was significantly higher (twice as high or 0.7 mg) at the edge of Pn2 compared to the interior (Table 5.6).

Amounts of EC decreased steadily over all distances in both forest types (Fig. 5.3d). Loss of EC was significantly higher at the oak edges compared to the interiors (Table 5.6). Loss of EC after 18 months was increased by 52 % (or 2.5 meq) and by 20 % (or 0.9 meq), respectively for Qr1 and Qr2. In the pine stands, EC amounts at the edge could be clearly distinguished from EC amounts in the interior (Fig. 5.3d). There was no significant effect of edge proximity on EC loss in the pine stands (Table 5.6).

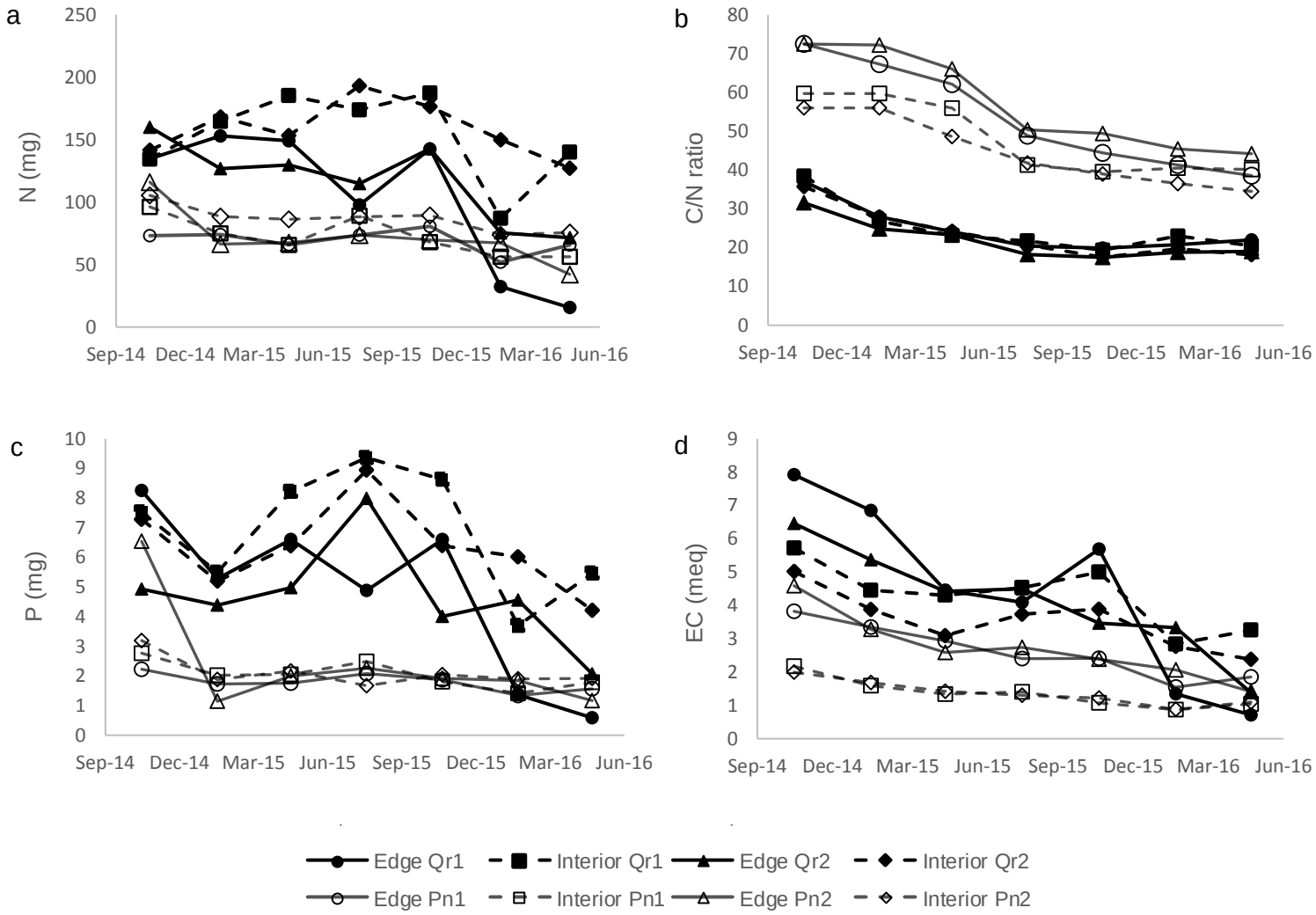

Fig. 5.3: Average absolute nutrient amounts in decomposing litter over time per litterbag at the edge (= 0 – 5 m) and interior (= 128 m) for a) nitrogen (N, in mg), b) C/N ratio, c) phosphorus (P, in mg) and d) exchangeable cations (EC, in meq) for litterbag series A (n = 2 per sampling moment).

Table 5.5: Absolute average changes in litter mass and releases of nutrient amounts in litterbags of series A after 18 months of litter decomposition (average of n = 2) in the oak (Qr1 and Qr2) and pine (Pn1 and Pn2) stands, relative changes (% of initial mass or amount) are presented between brackets. At 16 m of Qr2, only 1 litterbag was retrieved and omitted from the table due to disturbance by wild boars.

	Oak									
	Qr1					Qr2				
Distance (m)	Mass loss (g)	N (mg)	C (mg)	P (mg)	EC (meq)	Mass loss (g)	N (mg)	C (mg)	P (mg)	EC (meq)
Edge (0-5)	-9.3 (-93)	-119.8 (-89)	-4667.0 (-93)	-7.7 (-93)	-7.2 (-91)	-7.1 (-71)	-94.5 (-57)	-3689.2 (-73)	-2.9 (-58)	-5.1 (-78)
16	-5.8 (-58)	-41.2 (-31)	-3148.2 (-63)	-3.0 (-44)	-4.6 (-57)	-	-	-	-	-
64	-5.5 (-55)	-62.1 (-36)	-2868 (-57)	-5.2 (-59)	-4.0 (-63)	-6.0 (-60)	-47.6 (-31)	-3193.0 (-62)	-2.9 (-45)	-3.4 (-63)
Interior (128)	-4.4 (-44)	-0.02 (0)	-2402.5 (-46)	-2.1 (-27)	-2.5 (-43)	-5.4 (-54)	-18.6 (-13)	-2825.5 (-56)	-3.1 (-42)	-2.6 (-52)
	Pine									
	Pn1					Pn2				
Edge (0-5)	-5.3 (-53)	-9.5 (-13)	-2856.8 (-54)	-0.6 (-30)	-1.9 (-51)	-6.6 (-66)	-74.8 (-64)	-3500.7 (-66)	-5.4 (-82)	-3.2 (-69)
16	-6.1 (-61)	-31.0 (-36)	-3335.4 (-62)	-0.5 (-22)	-1.6 (-53)	-5.6 (-56)	-33.4 (-34)	-2936.9 (-55)	-2.3 (-59)	-1.7 (-64)
64	-5.0 (-50)	-5.5 (-7)	-2623.3 (-49)	-0.2 (-7)	-0.9 (-37)	-5.8 (-58)	-25.3 (-27)	-3091.8 (-58)	-1.3 (-42)	-1.2 (-57)
Interior (128)	-5.9 (-59)	-42.3 (-44)	-3092.1 (-59)	-1.0 (-35)	-1.0 (-47)	-5.3 (-53)	-32.5 (-31)	-2826.6 (-53)	-1.3 (-40)	-1.1 (-53)

Table 5.6: Effects of distance to the forest edge and time of sampling on mass loss, nutrient release of nitrogen (N), phosphorus (P), exchangeable cations (EC) and change in C/N ratio of decomposing litter in litterbags of series A over the whole period of 18 months. Bold values indicate significance ($p < 0.05$). The arrow indicates the direction of the effect of distance to the forest edge, i.e. increase with distance to the edge (↑) or decrease with distance to the edge (↓). Results of the Tukey HSD post-hoc tests are specified in case of a significant effect of distance to the forest edge.

	Oak							
	Qr1				Qr2			
	Distance to forest edge		Time	Interaction	Distance to forest edge		Time	Interaction
Mass loss	**0.001** ↓	0 > 16 = 64 = 128	**8.6 e-13**	0.096	**0.002** ↓	0 > 16, 64, 128	**6.7 e-14**	**0.013**
N loss	**5.92 e-13** ↓	0 > 16 > 64 > 128	**< 2 e-16**	**8.48 e-07**	**4.15 e-07** ↓	0 > 16 = 64 = 128	**2.10 e-07**	**0.008**
P loss	**1.43 e-12** ↓	0 > 16 = 64 > 128	**< 2 e-16**	**6.75 e-05**	**3.32 e-05** ↓	0 > 16 > 64 = 128	**8.06 e-09**	0.135
EC loss	**1.51 e-06** ↓	0 = 16 > 64 = 128	**1.32 e-12**	**0.0002**	**7.33 e-07** ↓	0 > 16 > 64 = 128	**1.47 e-10**	**0.005**
C/N change	0.227		**1.30 e-10**	**3.89 e-06**	**1.81 e-06** ↓	0 = 16 > 64 = 128	**3.13 e-14**	0.334
	Pine							
	Pn1				Pn2			
Mass loss	0.539		**3.3 e-12**	0.889	0.660		**< 2 e-16**	0.439
N loss	**0.0001** ↑	0 = 16 < 64 < 128	**0.001**	0.943	**8.22 e-08** ↓	0 > 16 > 64 < 128	**0.0004**	0.596
P loss	0.450		**0.007**	0.841	**4.55 e-10** ↓	0 > 16 > 64 = 128	**0.0035**	0.398
EC loss	0.316		**2.06 e-05**	0.987	0.079		**3.56 e-07**	0.493
C/N change	**2.89 e-07** ↑	0 = 16 < 64 < 128	**3.52 e-11**	0.464	**4.84 e-10** ↓	0 > 16 > 64 = 128	**1.12 e-08**	0.597

Pearson correlation analyses between litter mass loss after 18 months and soil physicochemical and environmental characteristics showed that in the oak stands litter mass loss was higher at higher pH values of mineral soil and at lower C/N ratios of the forest floor (respectively $p < 0.05$ for pHms and CNff, Table 5.7). The decomposition rate *k* of the oak stands was also positively correlated with the pH of the mineral soil ($p < 0.01$) and negatively correlated to the C/N ratio of the forest floor ($p < 0.05$, Table 5.7). Losses of N and EC after 18 months were negatively correlated to the C/N ratio of the forest floor in the oak stands ($p < 0.01$ and $p < 0.05$ respectively for N and EC, Table 5.7), while loss of P was positively correlated to pH of the mineral soil ($p < 0.05$, Table 5.7). In the pine stands, *k* was negatively correlated to the C/N ratio of the mineral soil and P loss was positively correlated with the C/N ratio of the forest floor ($p < 0.05$, Table 5.7).

Table 5.7: Pearson correlation coefficients between litter mass loss, decomposition parameter *k* and nutrient release of nitrogen (N), phosphorus (P) and exchangeable cations (EC), and change in C/N ratio of litter after 18 months of litter decomposition (litterbag series A), and soil physicochemical and environmental characteristics (pHms = pH of mineral soil, CNff = C/N ratio of forest floor, CNms = C/N ratio of mineral soil, Ndep = N deposition in kg N ha^{-1} yr^{-1}, soilT = soil temperature at a depth of 5 cm and soilM = soil moisture at a depth of 5 cm, n = 16) for the oak and the pine stands separately.

	Oak					
	Mass loss	*k*	N loss	P loss	EC loss	C/N change
pHms	0.741*	0.872**	0.687	0.723*	0.651	0.165
CNff	-0.802*	-0.745*	-0.843**	-0.648	-0.807*	-0.259
CNms	-0.172	-0.337	-0.059	-0.155	-0.019	0.308
Ndep	-0.301	-0.214	-0.187	-0.475	-0.262	0.065
soilT	0.385	0.567	0.371	0.269	0.451	0.596
soilM	-0.617	-0.947	-0.712	-0.561	-0.654	-0.972
	Pine					
pHms	-0.001	-0.549	0.041	0.504	0.445	0.211
CNff	0.337	-0.539	0.533	0.749*	0.630	0.565
CNms	0.056	-0.717*	0.225	0.482	0.582	0.155
Ndep	0.163	-0.252	0.111	0.262	0.328	0.141
soilT	0.469	-0.096	0.390	0.412	0.312	0.484
soilM	-0.901	-0.928	-0.683	-0.475	-0.307	-0.630

*p < 0.05, ** p < 0.01, *** p < 0.001.

5.3.2. Effect of litter quality, litter position and edge decomposer macrofauna on mass loss and nutrient release

Analysis of mass loss and nutrient release in series B and C was significantly affected by litter quality, litter position and the edge decomposer macrofauna (Fig. 5.4, Table 5.8). Averaged over the four stands, the temperature of the litter layer in the OTC was 0.73 (± 0.26) °C higher than outside the OTC. The temperature increase in the OTC was comparable to the soil temperature increase at the forest edge compared to the interior, as the temperature difference between edge (0 – 2 m) and interior (128 m) ranged between 0.42 and 1.28 °C at a depth of 5 cm in the mineral soil. In Qr1 and Pn1, litter quality, litter position and the interaction of litter quality and position, i.e. the interchange of edge and interior litter significantly affected mass loss (Table 5.8). In Qr1, edge litter lost more mass than interior litter, irrespective of its position, and litter at the edge lost more mass than litter in the interior or in OTC (Fig. 5.4a). Also in Qr2, litter at the edge lost more mass than litter in the interior (Fig. 5.4a), irrespective of its quality, but the effect was marginally insignificant (Table 5.8). In Pn1, edge litter lost more mass compared to interior litter and litter in the interior lost more mass than litter at the edge or in the OTC. No significant influence of litter position or quality was observed on mass loss in Pn2.

Loss of N was governed by litter quality in both oak stands and Pn2, where edge litter lost more N than interior litter (Fig. 5.4b, Table 5.8). In Qr1 and Pn1, litter position affected N loss in the same way as mass loss (i.e. litter at the edge lost more N than litter in the interior of Qr1, whereas in Pn1 litter in the interior lost more N than litter at the edge).

Edge litter lost more P than interior litter in Qr1 and Pn2 (Fig. 5.4c, Table 5.8). In both oak stands, litter at the edge lost more P than litter in the interior or OTC. In Pn1, the Tukey post-hoc test revealed that the interchange of litter significantly affected P loss, as P loss of interior litter in the interior differed from interior litter at the edge and P loss of edge litter at the edge differed from edge litter in the interior.

Edge litter of Qr1 and Pn2 lost more EC than interior litter in these stands (Fig. 5.4d, Table 5.8). In Qr1, litter at the edge lost significantly more EC than litter in the forest interior or OTC.

Relative changes in C/N ratios were significantly influenced by litter quality (Fig. 5.5, Table 5.8), with larger relative changes in C/N ratio in edge litter compared to interior litter in Qr2

and Pn2, but larger relative changes in C/N ratio in interior litter compared to edge litter in Pn1. In Pn2, litter at the edge had larger relative changes in C/N ratios than litter in the interior.

Table 5.8: Effects of litter position (edge, interior, OTC), litter quality (edge, interior) and their interaction on relative mass loss, nutrient release of nitrogen (N), phosphorus (P) and exchangeable cations (EC), and relative change in C/N ratio after 18 months of litter decomposition. Bold values are significant ($p < 0.05$). Results of the Tukey HSD post-hoc tests are specified: e = edge, i = interior.

Stand	Variable	Litter position		Litter quality		Interaction
Qr1	Mass loss	**3.07 e-6**	e > i	**0.004**	e > i	**0.013**
	N loss	**4.96 e-7**	e > i	**8.69 e-5**	e > i	0.554
	P loss	**1.61 e-5**	e > i	**0.0002**	e > i	0.057
	EC loss	**0.0004**	e > i	**0.0002**	e > i	0.459
	C/N change	0.388		0.394		0.417
Qr2	Mass loss	0.067		0.987		0.641
	N loss	0.142		**0.006**	e > i	0.091
	P loss	**0.001**	e > i	**0.027**	i > e	**0.021**
	EC loss	0.254		0.248		0.124
	C/N change	0.203		**0.0002**	e > i	0.057
Pn1	Mass loss	**0.020**	i > e	**0.028**	e > i	**0.022**
	N loss	**0.037**	i > e	0.791		**0.029**
	P loss	0.091		0.111		**0.004**
	EC loss	0.180		0.113		0.449
	C/N change	0.307		**0.009**	i > e	0.496
Pn2	Mass loss	0.438		0.177		0.224
	N loss	0.336		**0.036**	e > i	0.185
	P loss	0.084		**0.001**	e > i	0.100
	EC loss	0.612		**0.044**	e > i	0.763
	C/N change	**0.049**	e > i	**0.001**	e > i	0.059

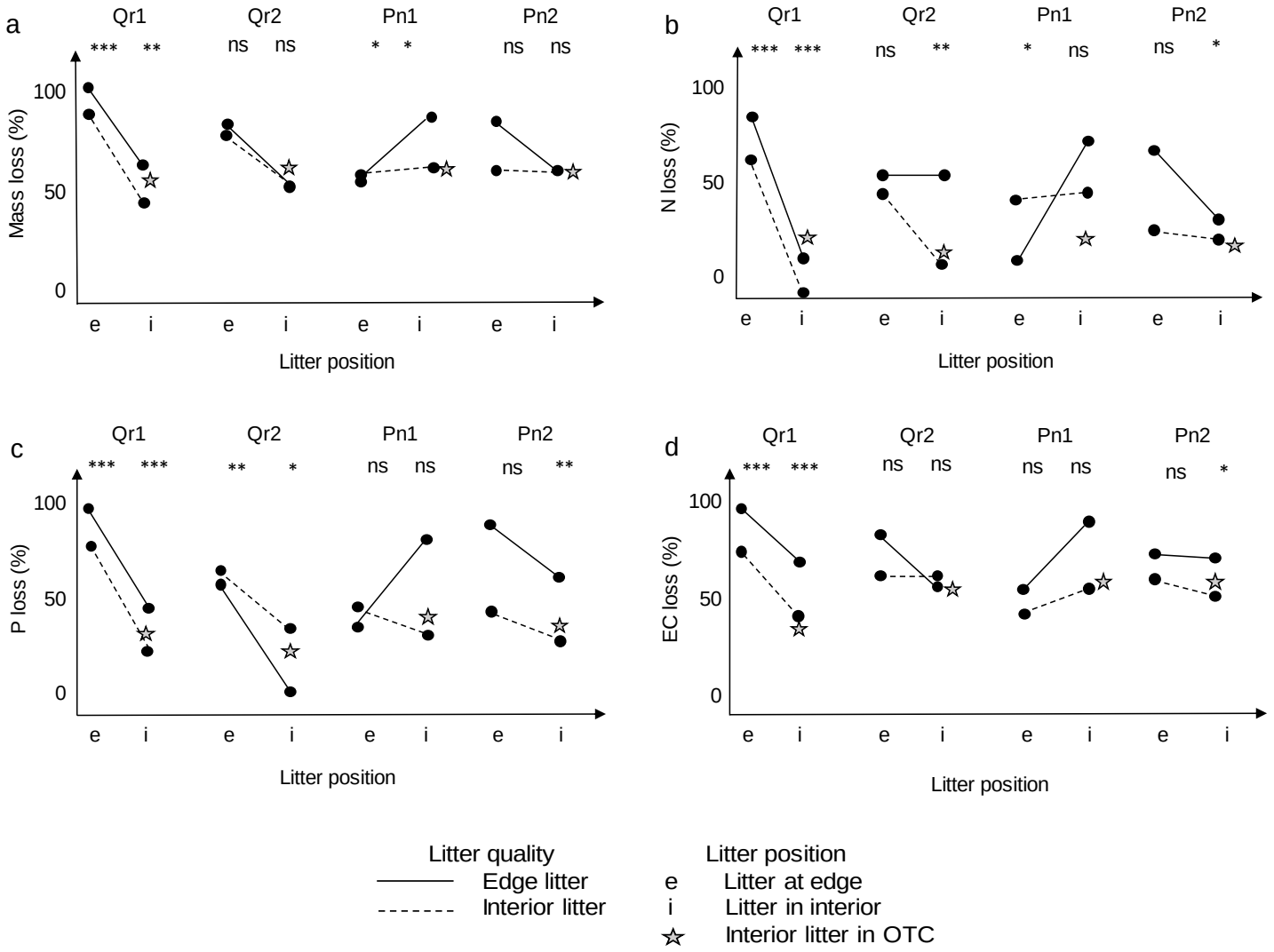

Fig. 5.4: Results of the two-way ANOVA's for litterbag series B and C showing the effect of litter position (e = edge, i = interior, ☆ = OTC; specified on the left) and litter quality (edge, interior; specified on the right) on litter mass loss and nutrient release of nitrogen (N), phosphorus (P) and exchangeable cations (EC) for the oak (Qr1 and Qr2) and pine stands (Pn1 and Pn2). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns= not significant.

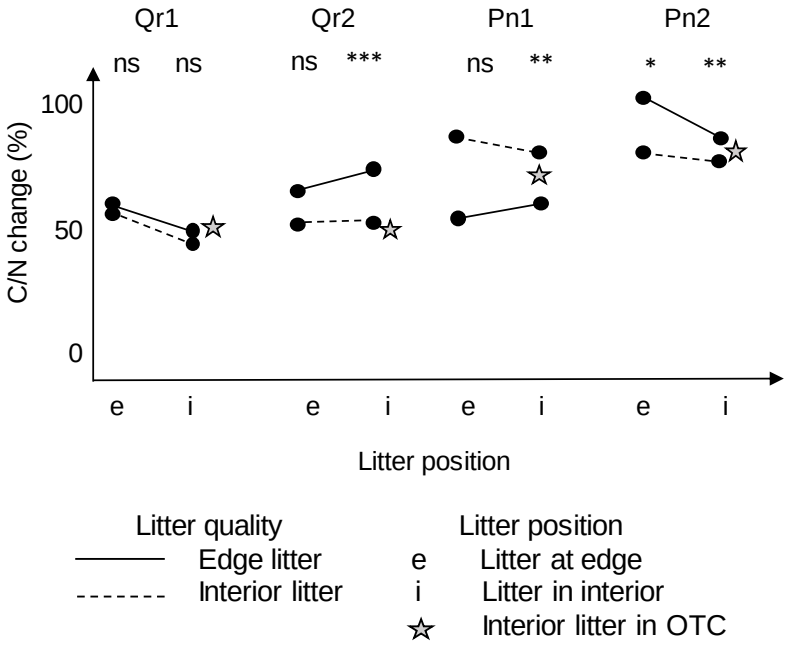

Fig. 5.5: Results of the two-way ANOVA's for litterbag series B and C showing the effect of litter position (e = edge, i = interior, ☆ = OTC; specified on the left) and litter quality (edge, interior; specified on the right) on the relative change in C/N ratio for the oak (Qr1 and Qr2) and pine stands (Pn1 and Pn2).). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns= not significant.

5.4. Discussion

In this study, we explored the edge effects on litter decomposition in four temperate forest stands in northern Belgium, Flanders, by monitoring litter mass loss, nutrient losses (N, P and EC) and C/N ratio along edge-to-interior transects during 18 months. The remaining litter mass was lower at the edge compared to the interior in the oak stands, but not in the pine stands. Nutrient release was higher at the edge compared to the interior for all nutrients in the oak stands, but only for N and EC in the pine stands. Furthermore, the roles of edge conditions (edge-specific microclimate, atmospheric deposition, soil fauna and soil physicochemical conditions), litter quality and edge decomposer community were investigated as underlying driving factors for litter decomposition at temperate forest edges by interchanging edge and interior litter (focusing on the influence of the edge conditions on the one hand and litter quality on the other hand) and placing litter in OTC (focusing on the role of the edge decomposer macrofauna). We found significant effects of the edge conditions, litter quality and the edge decomposer macrofauna on litter decomposition and nutrient release. In the next sections, the edge effect and its underlying driving factors on litter mass loss and nutrient release are discussed.

5.4.1 Litter mass loss along edge-to-interior transects

Our first hypothesis, stating faster litter decomposition at the edge compared to the interior, held true for the oak stands but not for the pine stands. Litter at the edge lost 87 % and 37 % more mass than litter in the interior, respectively in Qr1 and Qr2. In Qr2, relative remaining litter mass fluctuated in time along the edge-to-interior transects, probably due to the disturbance by wild boars (pers. obs.). Faster decomposition at the edge (0 - 50 m) was also observed in tropical forest fragments of 100 ha (Didham, 1998), but, in contrast, no edge effect on decomposition was observed in (sub)tropical forests (Vasconcelos and Laurance, 2005; Moreno et al., 2014) and even slower decomposition was found in the edge of mixed deciduous temperate forests in the UK (Riutta et al., 2012). Based on the data of this and previous studies, it seems that litter decomposition is not affected by a single general edge effect in all forests. Instead, the edge influence varies from site to site, probably resulting from the site-specific complex interaction of multiple factors involved in litter decomposition and affected by edge proximity (e.g. microclimate, atmospheric deposition, physicochemical soil conditions, decomposer community and litter quality). It is therefore important to identify the underlying driving factors for litter decomposition at forest edges.

Interchanging litter from edge and interior confirmed the influence of edge conditions on litter decomposition for Qr1, Pn1 and to a lesser extent also for Qr2. The pattern of relative remaining litter mass in the oak stands shows a ‘time lag’ of 6 months, after which the effect of edge conditions became apparent and larger litter mass losses were observed at the edge, irrespective of the litter origin (data not shown). This ‘time lag’ of 6 months coincides with the period of spring where temperatures and biological activity are rising (Baldrian et al., 2013).

We found no significant correlation between litter decomposition and soil moisture (Table 5.7), suggesting that soil moisture was not the driving factor for the edge effects. This is in contrast with the observation by Riutta et al. (2012) that litter decomposition was moisture-limited with slower decomposition at the drier forest edges. In agricultural soils, it is common practice to use RothC, developed by Coleman and Jenkinson (1996) to model the turnover of organic C in the topsoil. The model comprises a non-linear rate modifying factor for temperature and soil moisture. The lack of a correlation between mass loss and soil moisture/soil temperature in this study could have been due to the non-linear relationship between both types of variables.

Placing interior litter in OTC, creating a warmer 'edge' microclimate in the absence of the edge decomposer macrofauna, led to more remaining litter in one oak (Qr1) stand compared to interior litter at the edge (Fig 5.4a). Hence, in this stand, arthropod detritivores at the edge probably had a relatively large influence on the increased litter decomposition, as stated in our fourth hypothesis. It was also possible that the temperature in the OTC did not reach the temperature at the edge, resulting in lower decomposition rates. However, the relative increase in the litter layer temperature within the OTC fell within the range of the relative increase of the mineral soil temperature at the edge versus the interior. Many studies have shown an increased decomposition rate in the presence of macro-detritivores (Vasconcelos and Luizão, 2004; Slade and Riutta, 2012). Using two mesh sizes for their litterbags (5 mm to allow macrofauna and 1 mm to exclude macrofauna from the litterbags), Riutta et al. (2012) observed that the presence of macrofauna accelerated the decomposition rate (irrespective of soil moisture level or distance to the forest edge), and particularly for the oak leaves. Other studies, e.g. Hättenschwiler and Gasser (2005) have shown that the influence of soil fauna is strongest on the decomposition of recalcitrant litter types, such as pine and oak. The design of our litterbags was meant to minimize 'fall-through' of litter and still allow accessibility of soil macrofauna. Previous research in the same stands as our study, has shown an overall edge effect on woodlice and millipedes, with a higher abundance at the forest edge (De Smedt et al., 2016). They partly attributed their higher abundance due to more favourable soil chemical conditions (pH, exchangeable cations) at the forest edge, as higher abundances were found at higher pH values, higher Mg content and lower C/N ratio of the forest floor litter. The decomposer community could help explain the faster litter mass loss at our oak forest edges, as arthropod detritivores increase the accessible surface area of dead organic material for further breakdown by the microbial community (Harper et al., 2005).

Physicochemical soil conditions and litter stoichiometry influenced the litter decomposition in our forest stands. Correlation analyses revealed that litter mass loss was positively linked to the soil pH and negatively to the C/N ratio of the forest floor (litter and fermentation and humus layers) in the oak stands (Table 5.7). Indeed, the oak and pine stands were characterized by higher upper mineral soil pH values and lower forest floor C/N ratios at the edge than in the forest interior (Wuyts et al., 2011, 2013). The oak stands, characterized by lower lignin to N ratios and higher EC amounts than the pine stands, lost most litter mass at the edges. We also measured a negative relationship between the initial lignin concentration and litter mass loss in the pine stands, irrespective of the distance to the forest edge (Table

5.4). Faster decomposition rates have been observed for leaf litter of higher quality (low lignin and high nutrient concentrations) (Cornwell et al., 2008).

In our third hypothesis, we expected that if litter quality was the primary driver of litter decomposition, edge litter would lose more litter mass even in the forest interior. We observed this pattern in Qr1 and Pn1 (Fig 5.4a). However, the specific conditions of edge and interior (microclimate, atmospheric deposition, physicochemical soil conditions and decomposer community) were likely stronger regulators of litter mass loss than intraspecific variability in litter quality. For instance, in the pine forest Pn1, edge litter in the forest interior lost more mass compared to litter at the edge (Fig 5.4a). This could be due to the specific characteristics (such as understory vegetation, light availability and soil micro- and macrofauna) of the forest interior, which was relatively open, and edge, where the understory vegetation was dominated by grasses. In the study of De Smedt et al. (2016) this pine stand showed no decrease in millipede abundance and only a moderate decrease in woodlice abundance in function of distance to the forest edge, in contrast to the other investigated oak and pine stands (pers. comm.). This could indicate a less favourable microclimate at this forest edge and a more favourable forest interior (because of the open canopy) compared to the other sampled forest stands.

5.4.2. Nutrient release from litter along edge-to-interior transects

Nitrogen and P were released faster from decomposing litter at the forest edge than in the forest interior. Also EC release was higher at the edge, but only in the oak stands (Fig 5.3, Table 5.6). This confirms our first hypothesis. Generally, N and P dynamics in decomposing litter are characterized by an initial phase of leaching of soluble substances, followed by an increase in concentration (or immobilization phase), and a subsequent decrease (or mineralization phase) irrespective of distance to the forest edge (e.g. Berg and Staaf, 1981; Manzoni et al., 2008; Marklein et al., 2016). As the different litter compounds are not decomposed at the same rate, readily available nutrients decrease rapidly, while more recalcitrant compounds show a relative increase (Berg and McClaugherty, 2003). We observed the pattern of net N and P immobilization and release in the oak stands, but declining or constant N and P amounts in the pine stands, irrespective of distance to the forest edge (Fig 5.3a & 5.3c). The pattern of net N and P immobilization and release can be explained by the chemical composition of litter and the stoichiometric requirements of the microbial decomposer community that colonizes the litter during degradation (Jacob et al., 2009; Cline and Zak, 2014;). At low litter C/N ratios (N excess), homeostatic bacteria and fungi have a low nitrogen use efficiency (NUE), i.e. low immobilization and high N release

to the environment, but a high carbon use efficiency (CUE). In contrast, at high litter C/N ratios they are expected to lower their CUE while increasing their NUE (Sterner and Elser, 2002). The threshold elemental ratio (TER) expresses the ratio at which an ecological system switches from C limitation to nutrient limitation. Berg and McClaugherty (2003) proposed a TER ranging between 20 and 25 for the forest floor C/N ratio and between 20 to 30 for the litter C/N ratio based on a review of decomposition studies in temperate and boreal forests. During the first 6 months of the experiment, litter C/N ratio of the oak stands was higher than 25 (Fig 5.3b) and N may have limited microbial growth. After 6 months, oak litter C/N ratio was lower than 25 and consequently NUE decreased, leading to N release (Mooshammer et al., 2014). In the same way, the pattern of P release is negatively related to initial litter ratios of C/P and N/P (Jacob et al., 2009). Mooshammer et al. (2012) confirmed the negative relationship between P mineralization and litter C/P ratio for beech litter. We observed an uptake (immobilisation) of P between February 2015 and November 2015 in the oak stands, except at the edge (Fig 5.3c). Starting from December 2015, P was released at all distances, as P was probably no longer limiting microbial growth. In the pine stands, litter at the edge of Pn2 lost most N and P during the first 3 months (Fig 5.3a & 5.3c), probably via mechanical leaching of soluble compounds. During the remainder of the experiment and at other distances, N and P amounts decreased slowly (Fig 5.3a & 5.3c). Pine litter C/N ratios and C/P ratios were respectively above 25 (Fig 5.3b) and 1000 (data not shown) during the whole experiment, retarding decomposition. However, Manzoni et al. (2008) showed that decomposer communities are able to lower their CUE to obtain nutrients from recalcitrant substrates.

The release of P after 18 months was positively related to the pH of the mineral soil in the oak stands, which was higher at the forest edges compared to the interior and consequently could have favoured nutrient release. Nitrogen release was negatively correlated to the C/N ratio of the forest floor (litter and fermentation and humus layers) in the oak stands (Table 5.7), which was lower at the edges. Deciduous oak forests are characterized by lower forest floor and mineral topsoil C/N ratios than evergreen pine forests, due to the lower lignin and higher N content in oak leaves than in needles, which is preferred by soil macrofauna (David and Handa, 2010; Gerlach et al., 2013) and results in a faster breakdown and nutrient release (Cools et al., 2014). The higher initial EC concentrations in oak leaves compared to pine needles could be an extra stimulus for population development of soil macrodetritivores since these nutrients are essential components of the exoskeleton and haemolymph of soil macrofauna (Hopkin and Read, 1992; Kime, 1992). Vesterdal et al. (2008) found smaller C

pools in the organic horizon but larger C pools in mineral soils under tree species with low C/N ratios in leaf litter. Mueller et al. (2015) hypothesized that the negative effect of litter quality on organic horizon C stocks (via losses of litter derived C during decomposition) was counteracted by a positive effect of litter quality on mineral soil C stocks (via increased microbial CUE and subsequent retention of microbial-derived C in mineral soil). Our findings confirm their hypothesis and corroborate previous statements of Remy et al. (2016b, see Chapter 2, § 2.4.1.) and Wuyts et al. (2011), where faster litter degradation and transfer towards deeper soil layers was suggested as a possible explanation for lower N and C concentrations in the forest floor, but higher N and C concentrations in mineral soil at the edge compared to the interior.

The effect of the litter position (edge conditions) was apparent in the oak stands, where litter lost more N, P and EC at the edge of Qr1 and more P at the edge of Qr2 than litter in the interior (Table 5.8), confirming our second hypothesis (which stated that more nutrients would be released at the forest edge irrespective of litter origin). Nutrient release was also significantly affected by the OTC treatment in Qr1, as N, P and EC losses of interior litter at the edge were higher than interior litter in the OTC (Fig 5.4). Thus, the absence of the edge decomposer macrofauna significantly reduced nutrient release, as stated in our fourth hypothesis. The pine stand Pn1 behaved differently from the other stands, with higher losses of N when edge litter was placed in the forest interior compared to litter at the edge (Fig 5.4b). The pattern of nutrient release followed the observed pattern for litter mass loss in Pn1 (see § 5.4.1. for possible explanations).

Effects of litter quality were also measurable in Qr1 and Pn2, as edge litter lost more N, P and EC amounts than interior litter both at the edge and in the interior (Fig 5.4). Therefore, our third hypothesis, where the favourable litter quality of edge litter stimulates nutrient release irrespective of its location (edge/interior) held true in these stands.

5.5. Conclusion

Increased litter mass loss was observed at the forest edge compared to the interior in the oak stands, but not in the pine stands. Leaf litter mass loss was positively correlated to soil pH and negatively to the C/N ratio of the forest floor in the oak stands, but not in the pine stands. These favourable soil chemical conditions (higher pH values and higher atmospheric deposition of N and exchangeable cations (i.e. K^+, Mg^{2+}, Ca^{2+})) probably promoted the abundance of the decomposer macrofauna (woodlice, millipedes) and microfauna (see Chapter 4) at the edge, which may contribute to increased litter breakdown. Nutrient release was higher for all nutrients (N, P, EC) at the oak forest edges compared to the interior, but only for N and P in one pine stand and was also governed by the pH of mineral soil. We could confirm previous hypotheses of Mueller et al. (2015) and Remy et al. (2016b) that faster litter degradation at the edge lay at the base of lower C stocks in the organic layer and was counteracted by increased mineral soil C stocks (see Chapter 2). Litter position, litter quality and edge soil macrofauna all influenced litter decomposition and nutrient release, but the contribution of each driving factor depended on the specific edge characteristics of each site. Overall, we demonstrated an edge effect on litter decomposition and nutrient release, caused by the complex interplay of edge microclimate, atmospheric deposition, physicochemical soil characteristics, litter quality and soil decomposer community. Consequently, such edge effects must be taken into account when quantifying ecosystem processes, such as litter decomposition and nutrient cycling in highly fragmented landscapes, dominating Western Europe.

6. General discussion and conclusions

Due to a long history of land-use change, forest edges and small forest remnants have become important features in the European landscape (Decocq et al., 2016; Harper et al., 2005; Hofmeister et al., 2013). Despite several directives and policies aiming to reduce the use and emission of N, anthropogenic activities still give rise to high atmospheric N deposition levels. Enhanced N deposition in forests gives rise to eutrophication (Gundersen et al., 1998a, 1998b), acidification (De Schrijver et al., 1998) and species loss (De Schrijver et al., 2011). Forest edges are subject to higher atmospheric deposition compared to forest interiors (e.g. Devlaeminck et al., 2005; De Schrijver et al., 2007; Wuyts et al., 2008a, 2008b), potentially making them more prone to these negative effects. Increased N input via atmospheric pollution may disrupt the forest N cycle, as N saturated forests are characterized by increased N losses (Butterbach-Bahl et al., 2002; Gundersen et al., 2006). However, forest edges may challenge the current N-saturation paradigm, as Wuyts et al. (2011) measured a local decline in nitrate (NO_3^-) seepage within the first 20 m from the edge. As there were no significant differences in throughfall and leaching fluxes along the edge-to-interior transects, hydrology alone could not be the explanatory factor. They hypothesized that increased N retention, gaseous N emissions and dissolved organic N (DON) leaching in the soil are the main processes involved in the altered N cycle at the N enriched forest edge. However, their assumptions on the processes involved in the fate of N at forest edges needed thorough verification.

As previous studies suggested changes in microclimate, N deposition, N leaching and soil physicochemical characteristics at forest edges (e.g. Matlack, 1993; De Schrijver et al., 1998; Wuyts et al., 2008b), more insight into N and C cycling and sequestration is needed. Therefore, six temperate forest stands in northern Belgium (Flanders) and Denmark growing on acid, sandy quartz-dominated Podzols with a low base saturation were selected to deliver more insight in the mechanisms that drive the edge effect on the forest N cycle. All the forest edges bordered arable lands dominated by intensive livestock production (the main source of ammonia emission) and have experienced several decades of elevated N deposition. The selected forest edges comprised tree species relevant for their respective region, i.e. two oak (*Quercus robur* L., Qr1 and Qr2) and two pine (*Pinus nigra* ssp. *nigra* Arnold and *Pinus nigra* ssp. *laricio* Maire, Pn1 and Pn2) stands in Flanders and two spruce (*Picea sitchensis* and *Picea abies,* Ps and Pa) stands in Denmark. An overview of the stand and physicochemical characteristics can be found in Table 1.1.

In this chapter, a general and integrated discussion of the most relevant results of the four previous chapters is provided (§ 6.1) together with some implications for forest policy and global change mitigation (§ 6.2) and recommendations for future research (§ 6.3).

6.1. Main findings

The major aims of this thesis were (i) to assess the edge effect on N stocks, C stocks and their sequestration and (ii) to determine which processes of the forest N cycle differ between edge and interior. Therefore, different processes of the N cycle were investigated in Chapters 2, 3, 4 and 5, as shown in Fig. 1.8. The results of Chapters 2 to 5 are summarized in Fig. 6.1 and Table 6.1, where the relative increases/decreases of stocks and fluxes at the edge (0 - 20 m) compared to the interior are presented. In the following paragraphs, the edge effect on N deposition and microclimate, the N and C stocks and sequestration, the microbial community and the different processes of the N cycle is discussed.

Edge effect on N deposition and microclimate

Inputs of atmospheric N to forests often exceed that to low vegetation, such as grasslands and heathlands, due to the filtering effect of the canopy (Erisman and Draaijers, 2003; Bobbink et al., 2010). Moreover, forest edges are recognized as traps or 'hot spots' for atmospheric pollutants (Weathers et al., 2001). Erisman and Draaijers (2003) stated that there are two methods to estimate deposition: micrometeorological methods, used for dry deposition processes and throughfall. They estimated that dry deposition of acidifying compounds to forest edges in the Netherlands is increased by approximately 5 to 10%. In this thesis, N input into the forest ecosystem was quantified via N throughfall data of Wuyts et al. (2008b) and Ginzburg (2014). Nitrogen throughfall fluxes of the six selected stands were on average 34 % higher at the forest edge (0 - 20 m) compared to the interior (Fig. 6.1, Table 6.1). Ginzburg reflected that N throughfall data are likely an underestimation of the total atmospheric N input owing to the canopy uptake of atmospheric N and by neglecting N input by stem flow. However, stem flow constituted only 0.6 and 0.5 % of gross precipitation in temperate pine and oak stands in Mexico (Silva and Rodriguez, 2001). Although N input by stem flow was shown to be less than one percent of the throughfall N input in a Norway spruce stand (Christiansen et al*.,* 2006), increased wind turbulence at the edge (Saunders et al., 1991) may increase the contribution of N input by stem flow. Fenn et al. (2013) stated that the major limitation of throughfall deposition methods is the uncertainty of pollutant interactions with the canopy. Adriaenssens et al. (2011) measured foliar N uptake from wet

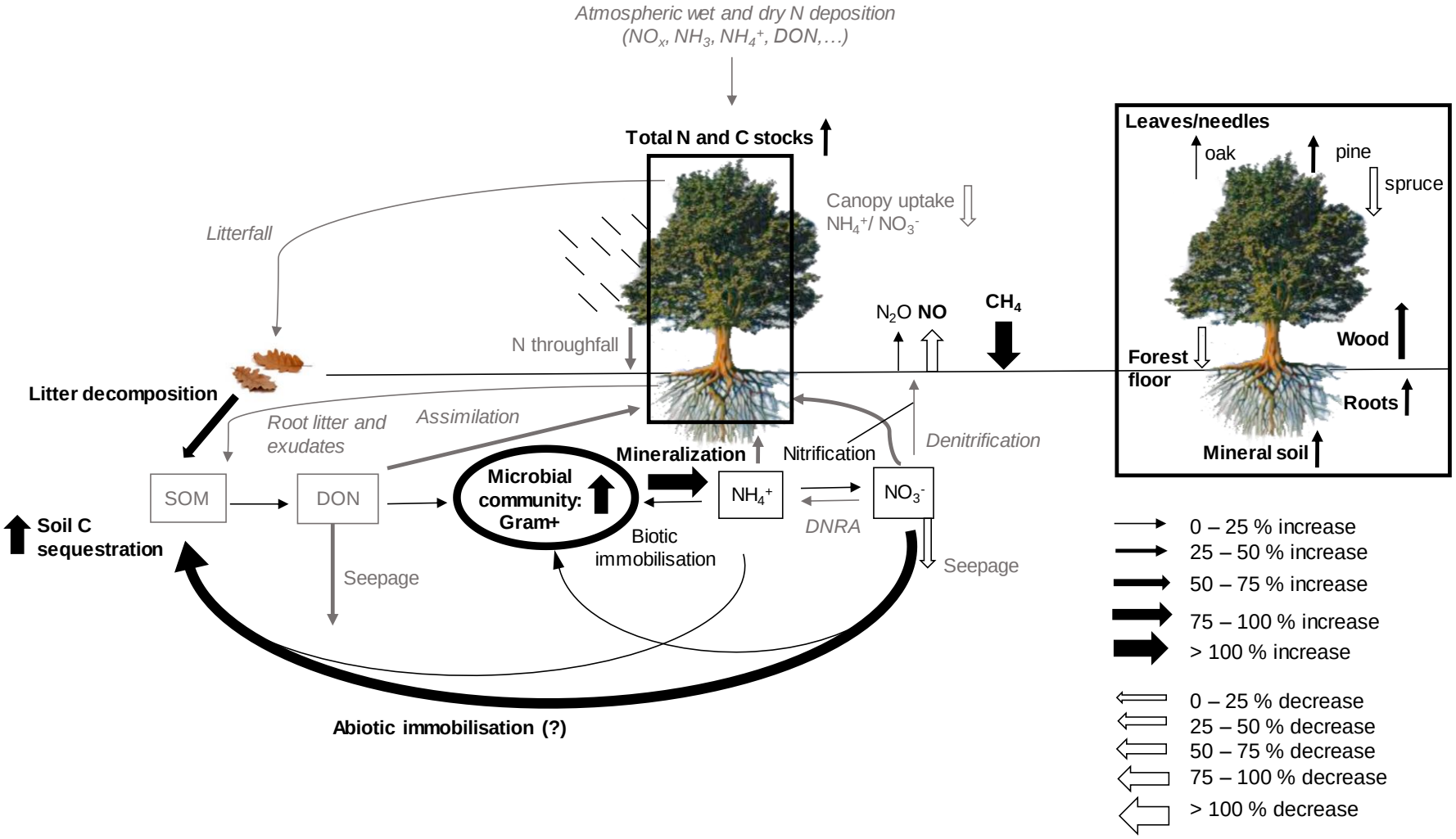

Fig. 6.1: Conceptual representation of the N cycle at the forest edge (0 – 20 m). Black: stocks and fluxes studied in this thesis, bold: stocks and fluxes studied in this thesis that differ between edge and interior, the relative difference is indicated by the thickness of the arrows (full = increase at the edge vs. interior, open = decrease at the edge vs. interior). Fluxes in grey were taken from other studies in the same forest stands (Sleutel et al., 2009; Wuyts et al. 2008b, 2011), fluxes in grey italic are assumptions based on our data and available literature. SOM stands for soil organic matter, DON for dissolved organic nitrogen, and DNRA for dissimilatory reduction of nitrate (NO_3^-) to ammonium (NH_4^+). Data on DNRA at forest edges are lacking. Differences in N and C stocks of the forest compartments and mineral soil at the edge vs. the interior are specified in the black box on the right.

Table 6.1: Overview of the mean relative increase (+) or decrease (-) (in %) of the different N and C stocks and fluxes at the edge (0 - 20 m) versus the interior. FH stands for the fermentation and humus layer, DON for dissolved organic nitrogen and DNRA for dissimilatory nitrate reduction to ammonium. The N and C stocks of leaves/needles are specified per forest type (o =oak, p = pine and s= spruce).

Variable	Mean relative increase (+) or decrease (-) at the edge vs. the interior (%)	Source
Total N deposition	+ 0 - 25	Erisman and Draaijers (2003)
N throughfall	+ 25 - 50	Wuyts et al. (2008b), Ginzburg (2014)
Canopy uptake	- 25 - 50	Wuyts et al. (2008b)
Total N and C stocks	+ 25 - 50	Remy et al. (2016b), Chapter 2
Leaves/needles	o: + 0 – 25, p: + 25 - 50, s: - 25 - 50	Remy et al. (2016b), Chapter 2
Wood	+ 50 - 75	Remy et al. (2016b), Chapter 2
Roots	+ 25 - 50	Remy et al. (2016b), Chapter 2
Forest floor	- 25 - 50	Remy et al. (2016b), Chapter 2
Mineral soil (0 - 30 cm)	+ 25 - 50	Remy et al. (2016b), Chapter 2
Soil C sequestration	+ > 100	Remy et al. (2016b), Chapter 2
Litterfall	+ 0 - 25	Remy et al. (2016b), Chapter 2
Litter decomposition	+ 50 - 75	Chapter 5
Root litter and exudates	+ 0 - 25	Remy et al. (2016b), Chapter 2
N assimilation in trees	+ 25 - 50	Remy et al. (2016b), Chapter 2
Biomass of Gram+ bacteria	+ > 100	Chapter 4
Mineralization	+ > 100	Chapter 4
Nitrification	0	Chapter 4
N retention		
Litter	- 50 - 75 (NH_4^+ and NO_3^-)	Chapter 4
FH	+ 50 - 75 (NH_4^+ and NO_3^-)	Chapter 4
Mineral soil (0 - 10 cm)	+ > 100 (only NO_3^-)	Chapter 4
Mineral soil (10 - 20 cm)	+ > 100 (only NO_3^-)	Chapter 4
NO_3^- seepage	- 25 - 50	Wuyts et al. (2011)
DON seepage	+ 25 - 50	Sleutel et al. (2009)
N_2O emission	0	Remy et al. (2016a), Chapter 3
NO emission	- 50 - 75	Remy et al. (2016a), Chapter 3
CH_4 uptake	+ > 100	Remy et al. (2016a), Chapter 3
Denitrification	0	Rich et al. (2003)
DNRA	-	no data

deposition for silver birch, European beech, pedunculate oak and Scots pine. Uptake rates for NH_4^+ were higher than uptake rates of NO_3^-, especially for the deciduous tree species (Adriaenssens et al., 2011). In remote or low polluted areas, several authors found lower NH_4^+ and NO_3^- fluxes in throughfall than in bulk precipitation, suggesting uptake of N by the canopy, whereas higher N fluxes in throughfall than in bulk precipitation have been reported at strongly polluted areas (Ignatova and Dambrine, 2000; Kristensen et al., 2004). Based on ion deposition fluxes in open field, at the edge and in the interior, measured during one year

by Wuyts et al. (2008b), the increase in N concentration during passage through the canopy could be calculated for the oak and pine stands in Belgium. The open field inorganic N (NH_4^+ and NO_3^-) deposition was on average 12.2 ± 0.7 kg N ha^{-1} yr^{-1}, while the edges of the oak and pine stands received on average 24.4 ± 8.3 kg N ha^{-1} yr^{-1} and 56.1 ± 6.5 kg N ha^{-1} yr^{-1}, respectively via throughfall. The interiors of the oak and pine stand received 20.4 ± 4.1 kg N ha^{-1} yr^{-1} and 35.3 ± 6.0 kg N ha^{-1} yr^{-1}, respectively via throughfall. As passage through the canopy increased N fluxes in throughfall (on average by 36 %), the canopy uptake of inorganic N at the edge was therefore set at a decreased rate of 30 to 60 % compared to the interior (Fig. 6.1, Table 6.1). Sleutel et al. (2009) also measured an increase in dissolved inorganic N (DIN) during passage through the forest canopy, which was highest at the forest edge (21.4 kg ha^{-1} yr^{-1} compared to 16.6 kg ha^{-1} yr^{-1} in the interior) while the ratio of dissolved organic C (DOC) and dissolved organic N (DON) substantially increased (from 2.1–2.4 in bulk precipitation to 11–16 below the canopy). They postulated that biogeochemical cycles are probably still influenced by the large historical N inputs, especially at forest edges where N deposition is increased compared to the interior.

Due to increased solar radiation, higher wind velocity and higher evapotranspiration rates, forest edges often have higher soil temperatures and lower soil and litter moisture contents than forest interiors (Marchand and Houle, 2006; Herbst et al., 2007; Schmidt et al., 2017). Soil temperature and moisture (at a depth of 5 cm) were monitored with a high temporal resolution (every 2 hours) along the edge-to-interior transects within the six selected forest stands. Along the transects in our well-developed forest edges small, but significant differences in soil temperature and moisture existed. Forest edges (0 - 2 m) tended to be warmer and drier than forest interiors (64 - 128 m), except for the spruce sites, which were wetter at the edge due to lower interception of rain. The contrasting microclimate at edge versus interior may affect cycling rates of soil organic matter (Conant et al., 2011) and contribute to differences in N and C stocks between forest edges and interiors.

Edge effect on N and C stocks

Total N and C stocks were increased at the forest edge compared to the interior, confirming the first research question. Total N and C stocks were respectively increased by 30 % and 43 %, when averaged over all forest types (Chapter 2). When looking at the different forest compartments, wood N and C stocks were on average increased by 56 %, root N and C stocks by 48 % and mineral soil N and C stocks by 30 % (Fig. 6.1, Table 6.1). Forest growth rates were not assessed in this thesis. However, due to the contrasting

microclimate at the edge (including increased solar radiation, higher soil temperatures, higher wind velocity, higher evapotranspiration rates,…) and increased availability of resources, such as light and nutrients, tree growth is likely enhanced at the edge compared to the forest interior (Reinmann and Hutyra, 2017). Increased growth rates at the edges would presumably coincide with increased nutrient uptake rates, increased N and C stocks and increased above- and belowground litter input. Indeed, coarse and fine root biomass (kg $tree^{-1}$) was on average 13 % higher at the forest edge compared to the interior, from which we assumed that root litter and exudates were also increased by 0 to 25 % at the edge (Fig. 6.1, Table 6.1). Increased root and wood N and C stocks at the forest edge were both affected by the higher stem density at the forest edges. Based on the increased root and stocks, we assumed that N uptake would be increased in the same order of magnitude (25 – 50 %, Fig. 6.1, Table 6.1). The favourable growth conditions at the edge were further confirmed by increased wood volumes (m^3 ha^{-1}) at the edge compared to the interior. The basal area (BA) along the edge-to-interior transects in this study was additionally calculated via the inventory of the diameter at breast height (dbh), but the BA was only significantly higher (37 %) at the edge (0 - 10 m) of one spruce stand (Ps) compared to the interior (80 – 130 m). Reinmann and Hutyra (2017) measured the BA of temperate oak forest fragments in southern New England (USA) and the basal area increment, which were on average increased by respectively 64 % and by 89 % at the edge (0 – 10 m) compared to a forest segment 20 to 30 m from the forest edge. In contrast, they observed no effect of edge proximity on total C and N content and root biomass in the top 10 cm of the soil. However, they also found that forest growth near the edge declined three times faster than that in the interior in response to heat stress during the growing season. Ziter et al. (2014) attributed constant aboveground C stocks in temperate forest fragments to the interplay of increased tree mortality at the edge due to abiotic stressors and increased tree productivity due to the contrasting microclimate compared to the forest interior. As the root biomass and wood volume in this thesis, were higher at the edge compared to the interior, we speculated that the studied forest edges are not (yet) subjected to negative effects of abiotic factors.

As N deposition is higher in coniferous forests compared to deciduous forests, a more pronounced edge effect on the pine and spruce stands than in the oak stands was expected. However, this was not the case in this study, signifying that **the edge effect is not solely driven by forest type**, but more likely the result of an interplay of several factors (landscape matrix, edge structure, height, age, leaf area index (LAI)). The N and C stocks in leaves were higher at the edges of the oak and pine stands, but not in the spruce stands due to

opposite patterns in LAI. The LAI was higher at the oak and pine forest edges, but lower in the spruce forest edges compared to the interior, probably due to stronger winds in Denmark, the shade tolerance of spruce trees and the management road in the forest interior (see § 2.4.1.). In all stands, the LAI was measured via hemispherical photographs, taken with the same lens (Sigma circular fisheye 8 mm f/4 EX DG) and analysed with the same software, i.e. Gap Light Analyzer (GLA version 2.0 1999) as Wuyts et al. (2008b) to avoid errors originating from different measuring techniques. However, hemispherical photograph-based measurements of LAI near forest edges are difficult due to the incoming light from the edge. Therefore, a smaller field of view was used and the LAI was integrated over the zenith angles of 0 to 60°.

In the forest floor, N and C stocks increased with distance to the edge, while N and C stocks decreased with distance to the edge in the mineral soil. Vesterdal et al. (2013) also measured proportional differences in C distribution under different temperate forests, with low C stocks in forest floors, where C stocks were high in mineral soil. We hypothesized that higher soil stocks at the edges could be linked to increased litter input (above- and belowground litter and root exudates) and faster litter degradation, due to microclimatic gradients, an increased growth rate and a different microbial and invertebrate abundance and community at the edge (De Smedt et al., 2016). Consequently, at the edge, less N and organic matter would be retained in the forest floor (i.e. the ectorganic layer) than in the forest interior. Wuyts et al. (2011) collected on average 7.5 ± 1.1 kg m^{-2} of FH layer at the edges (0 – 20 m) of the oak and pine stands, which was significantly lower than the 9.8 ± 0.4 kg m^{-2} of FH layer in the interiors of the oak stands and 11.0 ± 2.0 kg m^{-2} of FH layer in the interiors of the pine stands. Sleutel et al. (2009) also suggested a faster turnover of organic material at the forest edge of a Corsican pine stand as more DOM was released compared to the interior although there was less forest floor material at the edge position.

Edge effect on litter input and decomposition

The LAI was on average increased by 20 % in the first 10 m of the forest edges of the Belgian (oak and pine) stands and thus presumably also the flux of organic material to the forest floor. Consequently, litterfall at the edge was assumed also to be increased by 0 to 25 % compared to the interior in Fig. 6.1 and Table 6.1. It can be expected that the increased N deposition and altered microclimate at the edge not only stimulated LAI, but also earlier leaf development, earlier flowering and increased seed production. This could contribute to an altered and increased N and C input to the forest floor at the edge.

We could confirm the statements on increased litter degradation at the edge, as litter mass loss in the oak stands was on average 62 % higher at the forest edges versus the interior. The **increased litter mass loss and nutrient release observed at the oak forest edges compared to the interior** (Chapter 5) were governed by soil acidity and forest floor C/N ratio. In the pine stands, only release of N and exchangeable cations (EC, sum of Ca^{2+}, Mg^{2+} and K^{+}) was higher at the edge compared to the interior. A unique tripartite experimental set-up was used to determine the influence of the underlying driving factors litter quality, edge conditions (microclimate, atmospheric deposition, edge soil fauna and physicochemical properties) and edge arthropod detritivores on litter decomposition. The contribution of each driving factor depended on the specific characteristics of each forest edge. Litter quality mainly affected mass loss and nutrient release in both oak stands and one pine stand (Pn2), while litter position played an important role the second studied pine stand (Pn1), due to the relatively open forest interior. Arthropod detritivores had the strongest influence on litter mass loss and nutrient release in the oak stand Qr1. According to Reich et al. (2005) and Hobbie et al. (2006) higher soil temperatures and higher Ca^{2+} concentrations in litter increase the abundance of earthworms and forest floor turnover rates. In this thesis, mineral soil temperature and moisture (at a depth of 5 cm) were monitored during 2 years along the edge-to-interior transects. Oak and pine forest edges (0–2 m) tended to be warmer and drier than forest interiors (64–128 m). Although Riutta et al. (2012) demonstrated lower decomposition rates at forest edges due to moisture limitation, soil moisture was not a limiting factor for litter decomposition in this study. Moreover, earthworms were rare in the acid, sandy forest soils of this study. Instead, woodlice and millipedes were the dominant macrofauna present. These arthropods also benefit from high EC litter concentrations, as these are essential components of the exoskeleton and haemolymph (Hopkin and Read, 1992; Kime, 1992). De Smedt et al. (2016) found highest woodlice and millipede abundance in the first 7 m of the same forest edges.

Edge effect on microbial community and N cycling

The edge characteristics (warmer, higher pH, higher atmospheric deposition, lower forest floor C/N ratios, higher litter input) favoured the presence of arthropod detritivores (De Smedt et al., 2016), but also affected the microbial community. **Biomass of Gram+ bacteria was higher at the forest edges compared to the forest interiors** (Chapter 4), where the abundance of Gram+ bacteria was increased by 65 to 300 % at the forest edge (Fig. 6.1, Table 6.1). The higher abundance of microfauna probably contributed to the higher litter mass loss and nutrient release at the oak forest edges. Furthermore, the microbial

community structure was linked to mineralization and nitrification rates obtained via the *in situ* ^{15}N pool dilution technique (Chapter 4). **The higher biomass of Gram+ bacteria at the forest edges compared to the forest interiors was associated to higher mineralization rates. Gross mineralization rates were also stimulated at the warmer forest edges,** with an increase of 66 to 700 % at the edge (Fig. 6.1, Table 6.1). **Nitrification rates were not affected by edge proximity,** but the oak stand was characterized by higher nitrification rates than the pine and spruce stands. As mentioned above, the favourable edge conditions were expected to increase the flow of organic material via the forest floor to the mineral soil. Indeed, in this thesis, forest edges of all forest types (oak, pine and spruce) were characterized by higher mineralization rates compared to the interior. Additionally, recovery of ^{15}N via the ^{15}N tracing method in the edge and interior was assessed as a proxy for the long-term dynamics of the N cycle. In all forest types, **the forest interior retained more N in the litter layer, while N was stored in deeper soil layers at the edge**. After 10 months, retention of ^{15}N in the litter layer was on average 60 % and 63 % lower at the edge, respectively for $^{15}NH_4^+$ and $^{15}NO_3^-$, while retention in the FH layer was on average 64 % and 76 % higher at the edge, respectively for $^{15}NH_4^+$ and $^{15}NO_3^-$ (Table 6.1, separate soil layers not shown on Fig. 6.1). Retention of $^{15}NO_3^-$ in the mineral soil was substantially higher at the edge than in the forest interior (> 300 %), but this was not the case for $^{15}NH_4^+$. Wuyts et al. (2011) hypothesized that increased N retention was one of the main processes differing between forest edge and interior, next to gaseous N emissions and dissolved organic N (DON) leaching. Consequently, more N could potentially be adsorbed onto the organic matter and be retained in the soil (Kaiser and Zech 2000), which was measured for DON and dissolved organic C (DOC) by Vandenbruwane (2008) and Sleutel et al. (2009) for forest ecosystems under long-term N deposition on sandy soils in Flanders. The findings of Vandenbruwane (2008) indicated that microbial degradation was not the main mechanism responsible for the high retention of DOM, but physical sorption to the mineral soil probably was. Sleutel et al. (2009) measured increased DOC retention in the mineral soil (84 kg ha^{-1} yr^{-1} additional DOC retention) of a Corsican pine stand in the state forest of Ravels, Flanders (close to one of the Corsican pine stands of this study, Pn2) at the edge compared to the interior sites of their study. Adsorption of DOC to mineral soil material rich in iron or aluminum was suggested as the most important process responsible for C retention. As there was no correlation between N recovery and the living microbial biomass in this study, N retention could have occurred via increased abiotic immobilisation at the forest edges compared to the interiors. However, as recovery percentages were not completely reliable (sometimes negative or > 100 %) we do not want to emphasize this result. Moreover, the

sampled mineral soil of these sandy Podzols is characterized by low amounts of clay minerals. Thus, fixation onto clay minerals can be excluded as a major pathway of N retention. Therefore, the most likely stabilization mechanisms are biochemical stabilisation via the formation of recalcitrant phenolic compounds and microbial immobilisation.

Edge effect on N losses

Wuyts et al. (2011) measured that seepage of NO_3^- (at a depth of 90 cm) was decreased by 32 % at the edge (0 – 2 m) compared to the interior (64 – 128 m, Fig. 6.1, Table 6.1) of the oak and pine stands. In the oak stand Qr1, seepage of NO_3^- was measured at a depth of 30 cm due to the high ground water level. The spruce stands in Denmark (Ps and Pa) had low NO_3^- leaching losses that were sometimes even beneath the detection limit (< 0.01 mg/l), but in one of the spruce stands (Ps) NO_3^- leaching decreased with distance to the edge. Ginzburg (2014) attributed the low soil solution NO_3^- concentrations at the spruce stands to a high N retention capacity. Seepage of NH_4^+ was very low compared to seepage of NO_3^- (i.e. two orders of magnitude lower for the oak and pine stands) and was not indicated on Fig. 6.1. Data on DON seepage were taken from Sleutel et al. (2009), where DON leaching was 34 % higher at the forest edge of a Corsican pine stand (close to one of the Corsican pine stands of this study, Pn2) compared to the interior (Fig. 6.1, Table 6.1).

Forest edges also affected the fluxes of N and C trace gases, as fluxes of nitric oxide (NO) and methane (CH_4) differed between forest edge and interior, while the flux of nitrous oxide (N_2O) did not during our measuring campaign (Chapter 3). This edge effect was more pronounced in the oak than in the pine stand. **Forest edges emitted less NO and took up more CH_4** (on average respectively 60 % and 177 %) at the oak stand (Fig. 6.1, Table 6.1). Contrary to the postulated hypotheses, increased N deposition at the edges did not stimulate emission of NO or N_2O and did not inhibit uptake of CH_4 during the measurements in April and May 2014. Instead, **the contrasting microclimate at the forest edge influenced N and C trace gas fluxes as soil moisture variation between forest edge and interior was a key variable** explaining the magnitude of NO and CH_4 fluxes. Davidson et al. (2000) explained the trade-off between nitrification and denitrification in function of soil moisture, as in dry, well-aerated soil, the oxidative process of nitrification dominates, and the more oxidized gas, NO, is the most common nitrogen oxide emitted, while in wet soils, much of the NO is reduced before escaping the soil, and more N_2O is emitted. Nitrous oxide emissions have their optimum in the range of 70 to 80 % water-filled pore space (WFPS) depending on soil type (Davidson et al., 2000). However, WFPS values were never this high

in the studied stands and N_2O emissions were consequently low (< 10 µg m^{-2} h^{-1}), without any significant effect of edge proximity. Higher soil moisture values in the forest interior probably led to more optimal conditions for NO emissions. Methane uptake was favoured at the drier oak forest edge as beyond a soil moisture optimum more soil moisture will limit CH_4 diffusion (Wang and Ineson, 2003). Rich et al. (2003) studied communities of denitrifying bacteria from adjacent meadow and forest soils at two sites in Oregon (USA). They measured a significant decline in denitrifying enzyme activity at the transition of the meadows into the forest edges at both sites and an increase in denitrifying enzyme activity further away from the forest edge (30 to 40 m) at one site. The denitrification rate in Fig. 6.1 was set equal for edge and interior, but could be lower at the forest edge compared to the interior, due to the lower soil moisture levels.

Most studies on N and C trace gases measure several year-round fluxes to observe intra- and inter-annual fluctuations. However, as the aim was to get a first insight in the - up to now - unexplored edge effect on N and C trace gas fluxes, an explorative measurement campaign at high temporal resolution with continuous measurements (every two hours) was performed during two weeks in one oak and one pine stand (Qr2 and Pn2). Papen and Butterbach-bahl (1999) measured pronounced seasonal and interannual variations in N_2O emissions during a 3-year continuous measurement campaign in the Höglwald forest in Germany. Highest N_2O emissions were observed during frost periods and soil thawing, due to the high microbial N turnover rates in these periods. The edge effect on N and C trace gases will most likely also show interannual fluctuations, owing to the contrasting microclimate between edge and interior.

Edge effect on C sequestration

In Chapter 2, the link between the N and C cycle was made and the obtained soil C sequestration values were on average 155 % higher at the edge compared to the forest interior (Fig 6.1, Table 6.1). **The findings of this thesis underline the need to include forest edges in programs and models monitoring forest C changes, since substantial additional amounts of C can potentially be stored in forest edges.** De Vries et al. (2009) assumed that less C will be sequestered per unit N deposition with increasing N-enrichment. They estimated an average response of 5 to 35 kg C kg^{-1} N in soil, whereas the C sequestered per unit N deposition (C_{resp}) values of this study ranged between -8.2 and 27.3 kg C kg^{-1} N. The highest value was found in the interior of a spruce stand (Pa), which was characterized by moderate atmospheric N deposition levels. Reinmann and Hutyra (2017)

calculated the forest edge effect on aboveground C storage of the 2.2 million ha of forest that remains in southern New England (USA) and obtained an increase of 10 ± 1%. A drawback of their study is that they only considered aboveground C uptake, while forests of mid to high latitudes on the northern hemisphere store most of their C in the organic layer and mineral soil (Mol Dijkstra et al., 2009). Furthermore, they defined the first 10 m of the forest as the forest edge, while in most studies the forest edge effect is not receded within the first 10 m of the forest (e.g. De Schrijver et al., 2007; Devlaeminck et al., 2005, Wuyts et al., 2008b, 2009b). Therefore, the actual impact of the forest edge might be considerably underestimated in their study.

In summary, the edge characteristics (increased solar radiation, higher soil temperatures, higher pH, higher atmospheric deposition, lower forest floor C/N ratios, higher litter input) probably stimulated forest growth, increasing wood, root and mineral soil N and C stocks and soil C sequestration (Chapter 2). The favourable edge conditions stated above may have contributed to the increased abundance of arthropod detritivores (De Smedt et al., 2016) and Gram+ bacteria (Chapter 4). The soil macro- and microfauna at the edge stimulated N cycling processes, via increased decomposition rates in the oak stands (Chapter 5) and mineralization rates in all forest types (oak, pine and spruce, Chapter 4). Released N (mainly NO_3^-) was retained beneath the litter layer at the edge (Chapter 4), contributing to the high mineral soil N stocks at the edge compared to the interior. Furthermore, oak forest edges affected N and C cycles via a decreased emission of NO and an increased uptake of CH_4 (Chapter 3).

6.2. Implications for forest policy and global change mitigation

The studied forest edges stored large amounts of N and C (in above- and belowground biomass and soil) and showed increased N cycling rates, while the oak forest edges also emitted less NO and took up more CH_4 than forest interiors. However, it remains unclear for how long forest edges can sequester additional N and C under ongoing high N deposition.

In Chapter 2, we looked at a broader scale, by estimating the total forest N and C stock of Flanders (northern Belgium). De Schrijver et al. (2007) considered 58 % of the total forested area in Flanders as external forest edges, bordering a non-forested area, based on a digital forest cover map for Flanders (“Bosreferentielaag”, Aminal Afdeling Bos en Groen 2001) and a median forest edge distance of 50 m. When looking at the total (sum of biomass, forest floor and mineral soil until a depth of 30 cm) mean N and C stock in the forest interior,

excluding the influence of forest edge, we obtained 5.8 Mg ha^{-1} of N and 251 Mg ha^{-1} of C. However, the forest edge effect can be taken into account for Flanders via Eq. 6.1,

$$\% FI_{area} \; x \; FI_{stock}(Mg\ ha^{-1}) + \% FE_{area} \; x \; FE_{stock}(Mg\ ha^{-1}) \quad \text{(Eq. 6.1)}$$

where FI_{area} is the percentage of forest interior area in Flanders, FI_{stock} is the mean N (or C) stock of the forest interior (Mg ha^{-1}), FE_{area} is the percentage of forest edge area in Flanders and FE_{stock} is the mean N (or C) stock of the forest interior (Mg ha^{-1}). When including the edge effect, the mean total N and C stocks were respectively 7.5 Mg ha^{-1} of N and 365 Mg ha^{-1} of C showing an underestimation of respectively 22 % and 31 % when N and C stocks are calculated on regional scales based on data from forest interiors only. Even when considering a median forest edge distance of only 20 m, the mean N and C stocks were underestimated by 10 % and 15 %, respectively when calculating stocks based on data from forest interiors alone. Vande Walle et al. (2005) estimated the C stock of above- and belowground forest biomass in Flanders and obtained a mean C stock of 85.2 Mg ha^{-1} based on data of forest interiors. When subtracting the forest floor and mineral soil C stocks of the total mean C stock, we obtained a mean C stock of above- and belowground biomass of 138.1 Mg ha^{-1} in the forest interior, which is higher, but still in the same order of magnitude as the estimation of Vande Walle et al. (2005). Reinmann and Hutyra (2017) used the 2011 National Land Cover Database (NLCD) land cover maps (Homer et al., 2015) to scale up their data on C uptake to southern New England (USA) to stress the landscape-scale implications of the forest edge effect on growth and C storage in the temperate broadleaf forest. This region of the United States consists for 64 % of forest, from which 9.6 % is within 10 m of the forest edge. They calculated that the forest edge effect could increase aboveground C storage in southern New England by 10 % or from 81 Mg C ha^{-1} to 89 Mg C ha^{-1}.

On a global scale, both the ocean and terrestrial ecosystems remove a large fraction of anthropogenic emissions (Le Quéré et al., 2016) and soils are recognized as the major terrestrial C sink (Ogle and Paustian, 2005; Lal, 2008). Any significant change in the function of C sinks is of great importance to climate policymaking, as it affects the level of CO_2 remaining in the atmosphere. Vice versa, policy frameworks influencing land use and land use change could possibly trigger large changes in C storage capacity (Le Quéré, 2016). The size of the global forest C sink has increased together with rising atmospheric CO_2 and N deposition levels. However, a quantified understanding of how these drivers shape the forest C sink is lacking (Bellassen and Luyssaert, 2014). Pan et al. (2011) estimated a net global forest C sink of 1.1 ± 0.8 Pg C yr^{-1}, while Le Quéré et al. (2016) estimated the total

terrestrial sink to be 3.1 ± 0.9 Pg C yr^{-1}, both showing a rather large uncertainty. Contemporary data sets are needed to better constrain C cycle models and make future projections more accurate (Le Quéré et al., 2016). More robust and transparent data sets could be obtained via large-scale remote sensing, combined with frequent monitoring of local sites, such as the permanent plots of national forest inventories (Bellassen and Luyssaert, 2014). For instance, de Brogniez et al. (2015) created a map of the organic C content of the topsoil (0 – 20 cm) within the EU-25 (excluding Romania, Bulgaria and Croatia) by applying digital soil mapping techniques to a database arising from the Land use/Cover Area frame statistical Survey (LUCAS). The comparison of their map with the previous dataset on soil organic C (SOC) in Europe (Jones et al., 2005) underlined the influence of land cover on C content in soils. Furthermore, these maps provide policy-makers with baseline data for developing strategies for soil protection and may be used to estimate projected changes in SOC (Jones et al., 2005). De Brogniez et al. (2015) took into account forest type when mapping the organic C stock of the topsoil, but did not include edge effects in their study. We have shown that including edge effects in the calculation of C (and N) stocks can have a significant impact. Therefore, future maps and models aiming to estimate temperate forest C and N storage capacity should not ignore the edge effect but at least quantify the relative contribution of forest edges to the total forest area to avoid underestimations. Consequently, edge proximity (extracted from land cover maps) should be an additional variable in assessments of C and N stocks on national or regional landscape scales particularly for the European lowlands where the forests are fragmented and receive additional N from agricultural pollution.

Storage of C and N in forests is an important pathway in climate change mitigation (Nabuurs et al., 2007). However, as disruptions of internal forest N and C cycles arise from an increased concentration of atmospheric pollutants a more sustainable solution would be the reduction in the use and emission of polluting N and C compounds. The Flemish Institute for Technological Research (VITO) and the Flanders Environment Agency (VMM) calculated that the costs due to N pollution in Flanders ranged between 2.3 and 6.8 billion euro in 2009 alone. Recently (in 2014), the Flemish government agreed to implement a program for the reduction of N emissions (PAS), which will be fully operational in 2019. This should enable the conservation of natural habitats and wild fauna and flora within the Natura 2000 network, together with sustainable economic developments in agriculture, industry and traffic. As increased N deposition hampers the conservation of biodiverse natural habitats, reducing its negative effects is currently on the agenda of policy makers. This thesis aids in

understanding the consequences of increased N deposition on N and C cycling and sequestration in temperate forest edges.

6.3. Recommendations for future research

The experiments of this thesis were executed in temperate oak, pine and spruce stands in Flanders and Denmark growing on acid, sandy quartz-dominated Podzols. The conducted experiments could be repeated in forests comprising other tree species and growing on other soil types, to validate our findings. Acid, sandy soils are characterized by a low base saturation and are consequently more sensitive to acid buffering via Al^{3+} (De Schrijver et al., 2006). On richer soils with a higher cation exchange capacity and base saturation it will presumably take more time before discrepancies in the forest N and C cycle between edge and interior can be observed.

In this study, we sampled mineral soil to a depth of 30 cm. In quartz-dominated Podzols, the B horizon, rich in organic matter and oxides is typically below this depth. Therefore, our results only hold true for the upper soil layers of Podzols. Future research could focus on sampling the whole soil profile of Podzols, as potentially large C and (to a lesser extent) N stocks could be accumulated in the subsoil.

Reinmann and Hutyra (2017) also stated that forest age, forest type, and land cover adjacencies may be important factors in determining the magnitude of the forest edge effect. Firstly, the forest fragments studied here, and the forests of southern New England in their study are still relatively young (i.e. < 100 years old). Age-related decline in forest growth is a widely observed phenomenon, but the underlying mechanisms and timing of growth decline are complex (Smith, 2001), and it is unclear how forest edge growth rate and storage capacity will evolve with stand age. Secondly, all the forest edges in this thesis bordered arable lands dominated by intensive livestock production (i.e. high NH_3 emission) and have experienced several decades of elevated N deposition. Temperate forest edges in more remote areas and adjacent to other land use types will probably be characterized by a less severe edge effect.

Furthermore, additional research in temperate forest edges is needed to provide an adequate knowledge of their N and C storage capacity and long-term behavior. As forests play a role in climate change mitigation it is imperative that correct forest N and C budgets can be calculated. However, data are still lacking to obtain forest N and C budgets in which

edge effects can be incorporated. From Fig. 6.1, it is clear that for several fluxes of the N cycle the magnitude of the edge effect was roughly estimated. Therefore, additional measurements of litter fall, root litter and exudates, N assimilation and denitrification at forest edges are needed. To better understand the environmental impact of N and C trace gas fluxes from forest edges, additional and long-term measurements in other forest edges are necessary. To our knowledge, the process of dissimilatory nitrate reduction to ammonium (DNRA) has not been studied yet at forest edges. Our results indicated that the microbial community plays a major role in the altered N and C cycles at the edge. In this thesis, the microbial community structure was assessed via the extraction of phospholipid fatty acids (PLFA) and aminosugars (AS), as these techniques gave a fairly quick and broad overview of the microbial biomass present in the studied forest edges. However, a more detailed study of the microbial community at forest edges could be conducted by means of DNA sequencing. For instance, the data on N and C trace gases could be extended by looking into a specific subset of the microbial community, i.e. methane oxidizing bacteria, autotrophic and heterotrophic nitrifiers (bacteria, fungi and archaea), denitrifying bacteria and other N_2O oxidizing bacteria (owning an atypical nosZ enzyme system catalyzing the conversion of N_2O to N_2). Forest hydrology can also play a role in the N and C cycles. Data on throughfall and leaching fluxes showed no consistent increase or decrease along the edge-to-interior transects in this study. Therefore, gradients in N throughfall and leaching could not be attributed to hydrology alone. However, to obtain a full hydrological balance at the forest edge, data on transpiration, evaporation, stem flow, canopy storage and run-off are lacking.

Several authors (e.g. Devlaeminck et al., 2005; Wuyts et al., 2008a) measured increased deposition of the exchangeable cations (EC) Ca^{2+}, Mg^{2+} and K^{+} up to 50 m from the forest edge. We measured increased concentrations of EC in pine needles at the edge compared to the interior and high EC concentrations in the oak leaves. High nutrient concentrations have a stimulating effect on litter decomposition rates (Cornwell et al., 2008). However, it remains unclear what the effect of increased EC concentrations in the forest floor and mineral soil is on nutrient cycling at temperate forest edges.

Finally, we observed strong linkages between the N and C cycle at forest edges. However, N and C are also linked to P via stoichiometric relationships. Cleveland and Liptzin (2007) examined global-scale patterns of C:N:P ratios in soil and in soil microbial biomass and found well-constrained element ratios with significant variation between vegetation types

(i.e. forest vs. grassland). Based on our results, we assume that there is an edge effect on the P cycle in temperate forests under elevated N deposition.

Appendix

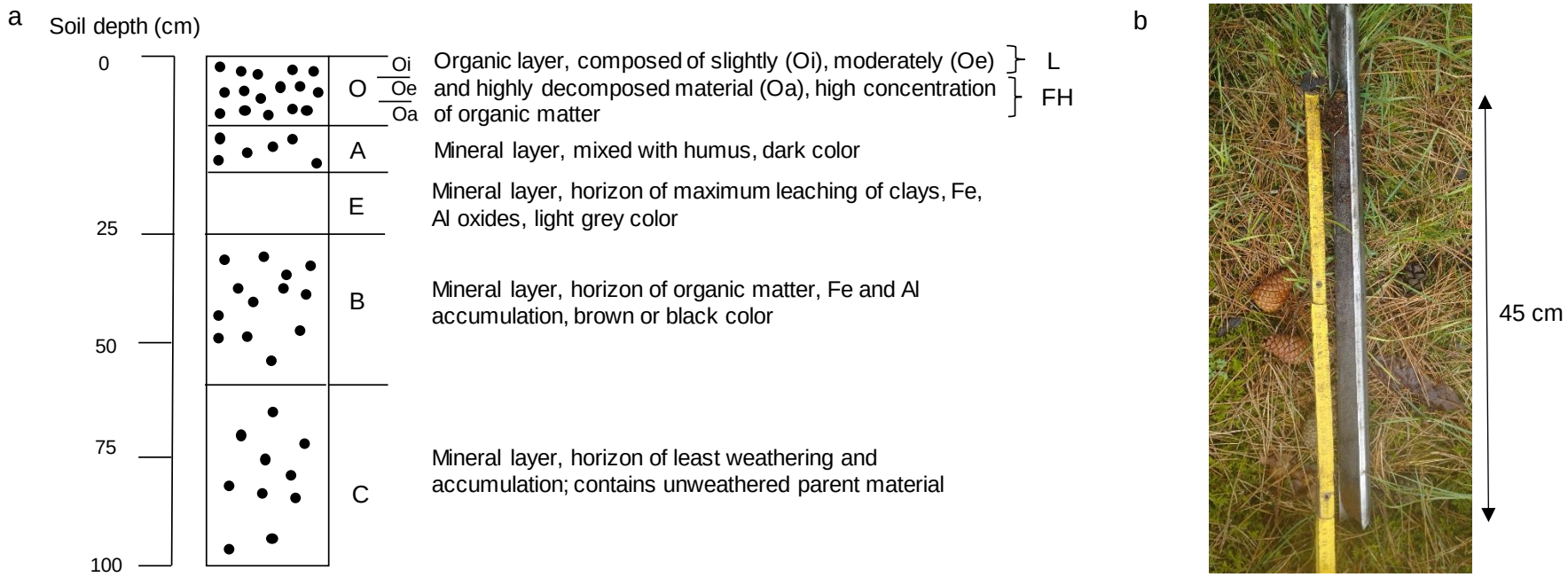

Fig. A-I: a) Schematic representation of the typical profile of a Podzol, showing the different horizons and the depth at which they occur. L stands for the litter layer and FH for the fermentation and humus layer, which is the terminology used in this study. Density of the black dots represents the concentration of organic matter (Chapin et al., 2002). b) Soil profile of the forest interior (64 m) of the pine stand Pn2.

Table A-I: Concentration of aluminium (Al^{3+}) in the Belgian oak (Qr1 and Qr2) and pine (Pn1 and Pn2) stands along the edge-to-interior transects within different mineral soil layers (0 – 5 cm, 5 – 10 cm and 10 – 30 cm). Standard deviations are presented between brackets (n = 3).

Al^{3+} concentration (meq kg^{-1})						
Oak						
	Qr1			Qr2		
Distance (m)	0 - 5 cm	5 - 10 cm	10 - 30 cm	0 - 5 cm	5 - 10 cm	10 - 30 cm
0	3.18 (4.43)	8.93 (8.45)	15.56 (10.03)	42.78 (7.71)	37.18 (7.11)	59.67 (6.20)
2	24.42 (4.97)	17.08 (3.00)	4.11 (2.06)	32.02 (8.48)	27.93 (8.81)	47.62 (1.13)
4	15.65 (5.85)	13.08 (2.73)	5.82 (4.34)	32.20 (2.83)	26.24 (3.74)	40.80 (1.06)
8	16.32 (1.32)	10.97 (2.11)	6.34 (1.06)	35.36 (3.28)	32.60 (2.33)	40.47 (3.14)
16	16.81 (2.60)	12.49 (0.51)	6.63 (1.11)	25.53 (3.17)	21.08 (0.93)	38.65 (4.33)
32	21.30 (4.83)	16.79 (2.61)	12.82 (1.51)	34.20 (3.16)	28.91 (3.97)	49.81 (5.56)
64	29.89 (7.33)	19.27 (1.30)	16.16 (2.02)	22.95 (4.86)	19.70 (9.29)	39.28 (6.72)
128	23.84 (2.67)	15.75 (1.33)	14.23 (2.90)	28.60 (7.01)	29.35 (5.79)	46.47 (2.02)
Pine						
	Pn1			Pn2		
Distance (m)	0 - 5 cm	5 - 10 cm	10 - 30 cm	0 - 5 cm	5 - 10 cm	10 - 30 cm
0	41.27 (4.89)	31.22 (3.35)	33.09 (1.58)	26.15 (4.17)	18.77 (4.16)	24.50 (12.55)
2	22.55 (5.85)	15.36 (4.93)	23.20 (3.95)	27.48 (3.27)	17.65 (2.54)	36.21 (3.44)
4	31.71 (8.85)	16.16 (3.05)	20.34 (2.32)	28.73 (1.60)	21.04 (3.68)	34.13 (2.98)
8	28.37 (4.09)	21.46 (8.38)	26.13 (5.10)	31.04 (6.25)	20.90 (4.81)	37.80 (8.50)
16	28.33 (3.36)	22.46 (0.29)	28.50 (7.74)	34.16 (6.59)	17.79 (6.57)	36.43 (4.37)
32	24.68 (8.08)	21.24 (10.36)	23.26 (11.83)	38.60 (1.82)	20.68 (7.40)	33.13 (10.20)
64	19.65 (2.46)	20.31 (5.74)	19.12 (1.79)	25.35 (4.88)	27.53 (6.88)	44.03 (7.29)
128	22.41 (4.26)	22.31 (3.52)	21.79 (2.61)	15.52 (7.30)	5.78 (4.55)	29.69 (1.64)

Table A-II: Phospholipid fatty acid (PLFA) and amino sugar (AS) concentrations (µg g^{-1} soil) in the oak, pine and spruce forests. Standard errors are presented between brackets.

		PLFA									AS							
Forest	Distance	Gram+		Gram-		Bacteria		Fungi		Ratio	Glu		Mur		Gal		GluMur	GluGal
Qr1	0	10.81	(0.32)	10.26	(0.89)	21.06	(0.99)	10.52	(0.48)	0.50	1252.13	(170.42)	54.23	(11.14)	542.13	(100.08)	23.09	2.31
	16	5.20	(0.28)	3.07	(0.41)	8.27	(0.55)	5.07	(0.83)	0.61								
	64	4.44	(0.13)	2.70	(0.19)	7.15	(0.26)	5.57	(0.36)	0.78	810.32	(61.39)	36.21	(1.61)	408.20	(68.41)	22.38	1.99
	128	2.97	(0.14)	2.11	(0.27)	5.09	(0.32)	2.97	(0.25)	0.58								
Qr2	0	4.49	(0.04)	2.45	(0.11)	6.93	(0.12)	3.03	(0.25)	0.44	1079.56	(73.89)	62.01	(5.73)	514.47	(47.34)	17.41	2.10
	16	2.63	(0.05)	1.43	(0.09)	4.06	(0.11)	2.12	(0.19)	0.52								
	64	1.98	(0.03)	1.34	(0.08)	3.31	(0.09)	1.66	(0.09)	0.50	363.43	(51.66)	34.68	(8.05)	177.71	(12.20)	10.48	2.05
	128	2.11	(0.04)	1.41	(0.08)	3.52	(0.09)	2.60	(0.09)	0.74								
Pn1	0	2.99	(0.11)	1.76	(0.16)	4.75	(0.22)	1.65	(0.12)	0.35	774.11	(51.26)	47.30	(3.59)	225.45	(18.51)	16.37	3.43
	16	2.95	(0.05)	1.62	(0.09)	4.57	(0.12)	1.78	(0.06)	0.39								
	64	2.29	(0.06)	1.30	(0.13)	3.59	(0.15)	1.15	(0.06)	0.32	787.79	(139.44)	44.39	(6.28)	326.82	(45.55)	17.75	2.41
	128	1.90	(0.07)	0.84	(0.10)	2.74	(0.14)	1.59	(0.31)	0.58								
Pn2	0	3.44	(0.04)	2.16	(0.07)	5.60	(0.09)	1.71	(0.12)	0.30	535.46	(24.86)	40.42	(4.56)	236.56	(23.64)	13.25	2.26
	16	2.62	(0.05)	1.66	(0.07)	4.27	(0.10)	1.48	(0.12)	0.35								
	64	1.75	(0.04)	1.00	(0.07)	2.75	(0.09)	1.17	(0.08)	0.42	447.59	(71.17)	33.66	(5.66)	194.30	(33.16)	13.30	2.30
	128	1.99	(0.04)	1.14	(0.08)	3.13	(0.10)	0.94	(0.05)	0.30								
Ps	0	1.66	(0.05)	0.69	(0.03)	2.35	(0.07)	1.05	(0.15)	0.45	244.88	(60.90)	10.43	(4.60)	96.15	(26.90)	23.47	2.55
	16	1.33	(0.02)	0.59	(0.03)	1.91	(0.04)	0.64	(0.02)	0.34								
	64	1.31	(0.01)	0.80	(0.03)	2.11	(0.03)	0.64	(0.06)	0.31	196.44	(52.50)	18.50	(5.08)	88.60	(31.14)	10.62	2.22
	128	1.75	(0.15)	1.40	(0.24)	3.14	(0.31)	1.28	(0.29)	0.41								

Glu = Glucosamine, Mur = Muramic acid, Gal = Galactosamine.

Table A-III: Mean $^{15}N_{rec}$ (%) values at the different times of sampling at the edge (0 – 5 m) and interior (64 m) of the oak (Qr2), pine (Pn2) and spruce forest (Ps). Standard errors are presented between brackets (n = 6).

			Oak				Pine				Spruce			
Layer	Treatment	Time	Edge		Interior		Edge		Interior		Edge		Interior	
L	$^{15}NH_4^+$	1	39.7	(7.3)	96.1	(11.8)	133.5	(43.6)	75.6	(23.2)	110.9	(50.9)	80.6	(6.2)
		2	15.2	(3.2)	44.7	(6.3)	78.5	(15.5)	44.6	(5.9)	149.4	(43.9)	75.3	(8.9)
		3	9.8	(1.2)	23.5	(6.3)	31.4	(6.7)	29.5	(8.0)	119.8	(18.5)	42.7	(3.3)
	$^{15}NO_3^-$	1	80.5	(9.8)	60.4	(8.5)	169.8	(39.6)	105.9	(34.1)	74.2	(28.3)	71.7	(7.4)
		2	16.7	(4.2)	9.2	(0.9)	65.0	(26.1)	78.1	(34.5)	66.2	(29.7)	62.7	(21.3)
		3	10.6	(2.0)	5.3	(1.9)	44.1	(12.6)	120.6	(11.6)	66.2	(11.5)	52.9	(8.4)
FH	$^{15}NH_4^+$	1	67.8	(35.2)	19.5	(1.9)	44.7	(15.3)	19.0	(6.8)	12.3	(3.2)	17.5	(5.2)
		2	27.4	(7.0)	10.3	(2.7)	28.6	(6.3)	11.4	(2.8)	20.3	(5.2)	22.5	(4.0)
		3	25.9	(5.6)	10.2	(2.2)	29.8	(12.4)	18.1	(5.0)	16.3	(2.8)	30.7	(6.8)
	$^{15}NO_3^-$	1	25.3	(2.3)	17.0	(4.4)	27.8	(8.2)	14.4	(3.8)	5.1	(0.5)	16.2	(4.8)
		2	17.1	(3.6)	9.2	(2.3)	17.0	(3.7)	14.1	(3.9)	13.9	(2.7)	22.7	(5.6)
		3	17.7	(4.0)	7.6	(3.1)	35.7	(11.6)	35.2	(4.9)	25.9	(3.6)	27.2	(4.6)
MS10	$^{15}NH_4^+$	1	-2.4	(3.1)	-5.0	(2.5)	-5.9	(3.4)	5.3	(2.8)	7.1	(2.6)	-1.2	(1.9)
		2	-3.5	(3.8)	-3.2	(2.2)	-4.5	(1.9)	2.9	(2.0)	13.0	(4.3)	2.0	(2.7)
		3	2.1	(4.9)	-4.9	(0.7)	2.3	(0.5)	3.7	(0.5)	10.2	(2.6)	5.8	(5.8)
	$^{15}NO_3^-$	1	4.5	(1.9)	-0.7	(1.4)	-4.4	(1.8)	1.2	(2.1)	-0.1	(2.0)	0.1	(0.9)
		2	10.0	(3.5)	-0.5	(0.6)	-1.1	(2.1)	1.8	(2.1)	9.7	(3.6)	20.5	(10.4)
		3	5.2	(1.1)	-3.2	(1.5)	-0.6	(0.4)	1.3	(0.8)	5.0	(5.3)	5.8	(5.4)
MS20	$^{15}NH_4^+$	1	-5.0	(2.4)	-3.1	(1.7)	-5.3	(1.7)	7.0	(1.2)	1.2	(1.6)	-1.1	(1.2)
		2	-2.6	(1.2)	-3.9	(2.0)	-3.4	(1.2)	3.8	(1.8)	6.0	(0.9)	3.2	(0.7)
		3	1.5	(3.5)	-2.6	(0.9)	-1.2	(0.9)	2.5	(1.5)	0.4	(0.7)	2.8	(2.2)
	$^{15}NO_3^-$	1	7.7	(3.6)	-1.5	(1.2)	0.0	(2.2)	3.6	(1.6)	0.1	(1.4)	-0.5	(0.7)
		2	11.1	(4.1)	-0.4	(1.4)	-0.3	(1.8)	6.9	(0.5)	3.5	(1.1)	3.2	(0.7)
		3	8.2	(2.1)	-0.9	(0.7)	-0.8	(0.9)	7.9	(4.0)	3.5	(2.0)	0.1	(1.0)

L = Litter, FH = fermentation and humus layer, MS10 = Mineral soil 0 - 10 cm, MS20 = Mineral soil 10 - 20 cm; Time: 1 = 1 day, 2 = 1 month, 3 = 10 months.

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Curriculum vitae

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Scientific publications

Publications in international journals with peer review cited in the Science Citation Index (IF = impact factor for 2017)

Remy E., Wuyts K., Boeckx P., Verheyen K. Driving factors behind litter decomposition and nutrient release at temperate forest edges (submitted to Ecosystems).

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Scientific activities

Participation in symposia with oral presentation

Remy E., Wuyts K., Boeckx P., Verheyen K. Nitrogen cycling and sequestration in temperate forest edges. Oral presentation at the *6th ICP Forests Scientific Conference*, 16-17 May 2017, Bucharest, Romania.

Remy E., Wuyts K., Boeckx P., Verheyen K. Nitrogen cycling and sequestration in temperate forest edges. Oral presentation at the *Joint European Stable Isotope User group Meeting (Jesium)*, 4-9 September 2016, Ghent, Belgium

Remy E., Wuyts K., Boeckx P., Verheyen. Nitrogen cycling and sequestration in temperate forest edges. Oral presentation at the *5th international Ecosummit on ecological sustainability: engineering change,* 29 August – 1 September 2016, Montpellier, France.

Remy E., Wuyts K., Boeckx P., Ginzburg S., Gundersen P., Demey A., Van Den Bulcke J., Van Acker J., Verheyen K. (2016) Strong gradients in nitrogen and carbon stocks in temperate forest edges. Accepted oral presentation at the *IUFRO all division 7 Conference on Global Change and Forest Health*, 25-29 April 2016, Istanbul, Turkey. Conference cancelled due to safety reasons.

Remy E., Wuyts K., Boeckx P., Verheyen K. Nitrogen cycling and sequestration in temperate forest edges. Oral presentation at the *PhD day of young soil scientists*, 26 February 2014, Brussels, Belgium

Participation in symposia with poster presentation

Remy E., Wuyts K., Boeckx P., Verheyen K. (2014) Nitrogen cycling and sequestration in temperate forest edges. Poster presentation at Annual Meeting of Benelux Association of Stable Isotope Scientists (BASIS), 27-28 March 2014, Nijmegen, The Netherlands

Participation in symposia

25-26 April 2013	Annual Meeting of Benelux Association of Stable Isotope Scientists (BASIS), Ghent, Belgium
26-27 March 2015	Annual Meeting of Benelux Association of Stable Isotope Scientists (BASIS), Utrecht, The Netherlands

Participation in specialist courses

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17-21 February 2014	International doctoral course “Using stable isotopes in research on forest ecosystems” (SIFER), Nancy, France

Supervision of intern students

February – June 2016	Matthias Minnebo: Microbiële gemeenschap van Vlaamse en Deense bosranden – Analyse met GC-MS van fosfolipiden en aminosuikers in bodemstalen