

Impact of nitrogen deposition on the functioning of heathland ecosystems

Kumulative Dissertationsschrift zur Erlangung des akademischen Grades
Doktor der Naturwissenschaften (Dr. rer. nat.)



angefertigt am
Institut für Ökologie
der Fakultät Nachhaltigkeit
der Leuphana Universität Lüneburg

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Eigenleistung

Die folgende Tabelle stellt die Beiträge der jeweiligen Autoren für die dieser Arbeit zugrunde liegenden Zeitschriftenartikel dar. Dabei setzen sich die verwendeten Kürzel aus den Anfangsbuchstaben der Autoren zusammen. Die Autoren sind entsprechend ihres Arbeitsanteils angeordnet. Bei gleichen Anteilen wurde die Reihenfolge alphabetisch angegeben.

Beitrag	I	II	III	IV	V
Untersuchungsdesign	SAP, AM, WH	KF, UF, WH, GvO, HM,	UF, WH, GvO, WUK	KF, WH, UF, GvO	UF, WH, GvO, WUK,
Datenerhebung	GvO, KF, UF, AM, AK, NB	UF, KF, KM, KMi	UF, CD, KSe	KF, UF, EB, SS, TM, TN, HM,	UF, MSW, KS,
Analyse	GvO, SAP, KF, UF, AM, AK, WH	UF, KF, WH	UF	UF; KF	UF
Verfassen des Manuskriptes	GvO, SAP, KF, WH	KF, UF, WH	UF, WH	UF, KF, WH	UF, WH

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Zusammenfassung

Atmogene Deposition des Stickstoffs (N) zählt neben Landnutzungsänderungen und dem Klimawandel zu den Haupttriebkraften des weltweiten Rückgangs biologischer Vielfalt in terrestrischen Ökosystemen (Sala et al. 2000). Die vorliegende Arbeit beschäftigt sich deshalb mit den Auswirkungen von N-Depositionen auf Ökosysteme und stellt trockene Sandheiden in den Mittelpunkt der durchgeführten Untersuchungen.

In den letzten Jahrzehnten ist eine zunehmende Vergrasung von Heideflächen zu beobachten, die als eine Folge chronischer N-Deposition betrachtet werden kann (Aerts & Bobbink 1999). Dennoch gelten Auslöser und Hintergründe der dabei ablaufenden Prozesse als nicht vollständig geklärt. Vor allem bei der in trockenen Sandheiden stattfindenden Verdrängung der ursprünglich dominanten Besenheide (*Calluna vulgaris*) durch das Gewöhnliche Pfeifengras (*Molinia caerulea*) bestehen noch Verständnislücken. Eine wichtige Frage ist, inwieweit anhaltend hohe N-Einträge dazu geführt haben, dass das Pflanzenwachstum in trockenen Sandheiden nicht mehr durch die Verfügbarkeit von Stickstoff, sondern durch die Verfügbarkeit von Phosphor (P) begrenzt wird. Des Weiteren stellt sich in diesem Zusammenhang die Frage, inwieweit ein solcher Wechsel der Nährstofflimitierung ein entscheidender Einflussfaktor bei dem zu beobachtenden Artenwechsel ist. Zudem wird angenommen, dass die Phase der Wiederbesiedlung von Heideflächen („Pionierphase“ im Sinne von Gimmingham 1972), wie sie sich in den ersten Jahren nach intensiven Pflegemaßnahmen wie dem Plaggen großflächig einstellt, ebenfalls eine bedeutende Rolle spielt. In Freiland- und Gewächshausversuchen wurde die Art der Nährstofflimitierung für *Calluna vulgaris* und *Molinia caerulea* anhand der Wachstumsreaktion gegenüber einer vollfaktoriellen Kombination aus N- und P-Düngegaben festgestellt (Beitrag I und II). Im Rahmen eines im Gewächshaus durchgeführten Konkurrenzversuches (Mono- vs. Mischkulturen) wurden zudem die Bedingungen der Pionierphase nach dem Plaggen simuliert. Dabei wurde der Einfluss von N-Einträgen auf das Konkurrenzverhältnis zwischen einjährigen Pflanzen von *Calluna vulgaris* und *Molinia caerulea* untersucht (Beitrag III).

Ein weiterer Schwerpunkt der Arbeit zielte auf den Verbleib von N-Einträgen in trockenen Sandheiden, verbunden mit der Frage, welcher Grad der N-Sättigung für diese Systeme festzustellen ist. Im Rahmen eines ¹⁵N-Markierungsversuches im Freiland wurde die räumliche und zeitliche Verteilung des applizierten stabilen Isotops ¹⁵N im Boden (O-, A- und B-Horizont) und in der Biomasse (aktuelle, sowie ein bis zwei Jahre alte Triebe von *Calluna vulgaris*, Mooschicht) untersucht. Zusätzlich wurden die ¹⁵N-Verluste durch Sickerwasseraustrag ermittelt (Beitrag IV).

In den kommenden Jahren werden nicht nur die N-Deposition, sondern auch der Klimawandel einen entscheidenden Einfluss auf die Artenzusammensetzung von Heiden haben. Insbesondere die Wirkung möglicher Interaktionseffekte aus diesen beiden Einflussgrößen gilt bisher weitgehend als ungeklärt. In der vorliegenden Arbeit wurde deshalb den kombinierten Wirkungen aus N-Deposition und Sommerdürren (deren zukünftige Zunahme im Rahmen des Klimawandels prognostiziert wird) Rechnung getragen. In einem Gewächshausversuch wurde die Reaktion von *Molinia caerulea* auf N- und P-Düngegaben sowie auf Sommerdürren untersucht. Analysiert wurden dabei Biomassen- und Nährstoffallokationsmuster (N, P) sowie ¹⁵N-Verteilungsmuster nach Applikation eines ¹⁵N-Markers (Beitrag V).

Die Ergebnisse der Düngeversuche im Freiland zeigten, dass sowohl die Biomassenproduktion von *Calluna vulgaris* (Beitrag I) als auch von *Molinia caerulea* (Beitrag II) in Reaktion auf die N-Düngung zunahm. Während des durch eine ausgedehnte Trockenperiode gekennzeichneten Sommers in 2006 zeigte sich jedoch für *Molinia caerulea* im Freiland eine Zunahme nach P-Düngung. Insgesamt ist für beide Arten dennoch von einer immer noch überwiegenden Limitierung des Wachstums durch Stickstoff auszugehen. Im Rahmen des durchgeführten Konkurrenzversuches (Beitrag III) war für *Calluna vulgaris* und für *Molinia caerulea* in Monokultur ein Biomassenzuwachs in Folge der N-Düngung festzustellen. Darüber hinaus zeigte sich eine Verschiebung des Spross-Wurzel-Verhältnisses zu Gunsten der oberirdischen Biomasse bei beiden Arten, besonders aber für *Calluna vulgaris*. In Mischkulturen nahm *Molinia caerulea* mehr als 65% des verabreichten Stickstoffs auf. Die Biomasse von *Calluna vulgaris* war in Mischkulturen im Vergleich zu Monokulturen auf die Hälfte reduziert. Dieses Ergebnis kann auf eine schlechtere N-Versorgung unter Konkurrenz mit *Molinia caerulea* zurückgeführt werden. Demnach ist anzunehmen, dass beim Vorkommen beider Arten unter Konkurrenz nur *Molinia caerulea* von N-Einträgen profitiert. Wachstumszunahmen von *Molinia caerulea* aufgrund einer höheren N-Verfügbarkeit sollten schließlich zur Dominanz gegenüber *Calluna vulgaris* führen, und damit den zu beobachtenden Artenwechsel während der hier simulierten Pionierphase einleiten.

Im Rahmen des ¹⁵N-Markierungsversuches (Beitrag IV) zeigte sich, dass nach zwei Vegetationsperioden der größte Teil des ¹⁵N-Markers in Biomasse oder Boden festgelegt wurde und weniger als 0,05% dem System über das Sickerwasser verloren gingen. Als Senke des ersten Jahres war vor allem die Moosschicht von Bedeutung mit einer ¹⁵N-Wiederfindung von 64%, gefolgt von der organischen Auflage des Bodens (18%). Die Biomasse von *Calluna vulgaris* (Summe aus aktuellen und ein- bis zweijährigen Trieben) wies nur eine geringe ¹⁵N-Wiederfindung auf (<3%), was auf ein hohes N-Immobilisationspotenzial des O-Horizontes hindeutet. Während in der Moosschicht im

zweiten Jahr 23% weniger ^{15}N wiedergefunden wurde, gewann die Summe der Bodenkompimente 11% hinzu. Es wird angenommen, dass der Boden, insbesondere der B-Horizont, langfristig als stabile Senke für N-Einträge dienen könnte. Die Ergebnisse zeigen zudem, dass die untersuchten Heiden trotz anhaltend hoher N-Depositionsbelastung immer noch einen geringen Grad der N-Sättigung aufweisen.

Die Ergebnisse des Gewächshausversuches mit *Molinia caerulea* unter einer vollfaktoriellen Kombination von Düngung (N und P) und Dürrebehandlung (Beitrag V) ergaben einen Anstieg der Biomassenproduktion unter N-Düngung, aber nur einen geringen Rückgang unter Dürre. Es fanden sich starke Interaktionseffekte bei Kombination beider Behandlungen vor allem in Form eines bis zu 10-fachen Anstiegs abgestorbener oberirdischer Biomasse. Der aufgrund der N-Düngung verursachte Zuwachs der oberirdischen Biomasse sollte einen erhöhten transpirationsbedingten Wasserbedarf zur Folge gehabt haben. Als dieser unter Dürre nicht mehr gedeckt werden konnte, führte dies zum Absterben von Biomasse. N-Konzentrationen sowie ^{15}N -Allokationsmuster zeigten einen hohen N-Verbleib in der abgestorbenen Biomasse auf, was zudem auf eine Störung der N-Resorption schließen lässt. Ein Anstieg der Häufigkeit von Sommerdürren in bereits durch N-Deposition belasteten Lebensräumen könnte somit die Konkurrenzfähigkeit von hochproduktiven Arten wie *Molinia caerulea* schwächen.

Bei gemeinsamer Betrachtung der Ergebnisse der unterschiedlichen Untersuchungen lässt sich feststellen, dass trotz jahrzehntelanger hoher N-Deposition sowohl das Wachstum von *Calluna vulgaris*, als auch von *Molinia caerulea* in den untersuchten Heiden überwiegend N-limitiert ist. Dieses Ergebnis geht einher mit der Feststellung, dass die untersuchten Heiden einen geringen Grad der N-Sättigung aufweisen. Das hohe ^{15}N -Immobilisationspotenzial des Bodens kann dabei als Erklärung für die weiterhin bestehende N-Limitierung dienen. Obwohl ein eindeutiger Wechsel der Nährstofflimitierung von Stickstoff zu Phosphor bisher noch nicht stattgefunden hat, zeigen die Ergebnisse des Konkurrenzversuches dennoch eine Konkurrenzüberlegenheit von *Molinia caerulea* gegenüber *Calluna vulgaris* unter verbesserter N-Versorgung. Die Bedingungen der hierbei simulierten Pionierphase unterscheiden sich jedoch von denen älterer Bestände wie sie im Rahmen des ^{15}N -Markierungsversuches untersucht wurden. In der Pionierphase (nach dem Plaggen) fehlt das N-Immobilisationspotenzial der organische Auflagen sowie der Moosschicht, so dass N-Einträge weitgehend durch die Pflanzen aufgenommen werden können. Unter diesen Bedingungen profitiert einzig *Molinia caerulea* von erhöhten N-Einträgen und kann *Calluna vulgaris* verdrängen, bevor diese eine geplaggte Fläche vollständig wiederbesiedeln kann. Inwieweit ein zunehmendes Auftreten von Dürreperioden im Zuge des Klimawandels zu einer Veränderung der Konkurrenzbedingungen zwischen *Calluna vulgaris* und *Molinia caerulea* führen wird, kann noch nicht hinreichend prognostiziert werden. Obwohl *Molinia*

caerulea bei N-Düngung kombiniert mit Dürrebehandlungen eine Wachstumsreduktion und Verluste durch das Absterben von Biomasse zeigte, sind ähnliche Reaktionen auch für *Calluna vulgaris* bekannt. Um die zukünftige Artenzusammensetzung in trockenen Sandheiden besser vorhersagen zu können, sind somit weitere Untersuchungen notwendig, die vor allem den Einfluss möglicher Interaktionseffekte zwischen Klimawandelfaktoren und N-Deposition auf die bestehenden Konkurrenzverhältnisse berücksichtigen.

Einleitung

Ursache und Wirkung von Stickstoffeinträgen

Die atmosphärischen Einträge von Stickstoff (N) haben sich seit Beginn der Industrialisierung bis Ende des 20. Jahrhunderts global betrachtet verdreifacht (Galloway et al. 2004). Anthropogen verursachte N-Emissionen sind dabei hauptsächlich auf eine intensive Landwirtschaft sowie auf die Verbrennung fossiler Brennstoffe zurückzuführen. Atmosphärische N-Einträge tragen zum einen zur Versauerung von Ökosystemen bei, zum anderen zu deren Eutrophierung (Hornung & Langan 1999). Es zeigen sich Veränderungen der Nährstoffkreisläufe und –bilanzen, eine Zunahme der pflanzlichen Biomassenproduktion und Einflüsse auf die bestehenden Konkurrenzverhältnisse. In vielen Ökosystemen sind letztendlich Veränderungen in der Artenzusammensetzung zu beobachten. Diese werden begleitet von einem Verlust von Arten sowie insgesamt einem Rückgang der Artenvielfalt (Bobbink et al. 1998; Bai et al. 2010; Maskell et al. 2010). Art und Ausmaß der Beeinträchtigungen durch N-Einträge hängen jedoch nicht nur von der Depositionsrate ab, welche räumlich stark variieren kann, sondern fallen auch für einzelne Ökosysteme unterschiedlich aus (Galloway et al. 2004).

Dem Minimumgesetz von Liebig zu Folge wird das Wachstum der Pflanzen stets durch die knappste Ressource limitiert. In den meisten terrestrischen Ökosystemen wird bei ausreichender Versorgung mit Wasser und Licht Stickstoff zum Mangelfaktor. Eine Zunahme der N-Verfügbarkeit führt somit unter N-Limitierung zu einer Zunahme der pflanzlichen Biomassenproduktion. Erst bei Sättigung des N-Bedarfs wird die relative Verfügbarkeit anderer wichtiger Mineralnährstoffe, meist Phosphor (P), entscheidend für die Biomassenproduktion (Vitousek & Howarth 1991; Verhoeven et al. 1996). Eine besondere Sensibilität gegenüber N-Einträgen ist demnach für solche Lebensräume festzustellen, die sich durch eine besondere Nährstoffarmut, insbesondere eine extrem niedrige N-Verfügbarkeit, auszeichnen (Bobbink et al. 1998). Nährstoffarme Ökosysteme von hohem Naturschutzwert, wie zum Beispiel Moore, Magerrasen und Heiden, sind somit am stärksten von den Auswirkungen der N-Deposition betroffen (Bobbink et al. 1998; Aerts & Bobbink 1999). Um Schutz und Erhalt dieser Lebensräume in Hinblick auf anhaltend hohe N-Einträge gewährleisten zu können, besteht jedoch noch Forschungsbedarf.

Die vorliegende Arbeit beschäftigt sich im Folgenden mit den Auswirkungen von N-Einträgen auf trockene Sandheiden.

Zwergstrauchheiden und ihre Beeinträchtigung durch Stickstoffeinträge

Zwergstrauchdominierte Offenlandschaften gehören zu den charakteristischen Kulturlandschaften Nordwest-Europas (Heil & Aerts 1993). Während sie lange Zeit vielerorts das Landschaftsbild bestimmten, ist ihre Verbreitung heute europaweit stark rückläufig. Ursache dafür sind vor allem weitreichende strukturelle Veränderungen in der landwirtschaftlichen Flächennutzung (Heil & Aerts 1993; Webb 1998). Aufgrund ihres Beitrags zur biologischen Vielfalt sind sie jedoch von hohem Wert für den Naturschutz (Ellenberg 1996). Sie werden gemäß Anhang I der FFH-Richtlinie („Fauna-Flora-Habitat-Richtlinie“, RL 92/43/EWG) zu den „natürlichen Lebensräumen von gemeinschaftlichem Interesse der Europäischen Union“ gezählt sowie zu den gesetzlich geschützten Biotopen gemäß Bundesnaturschutzgesetz (§ 30 Abs. 2 Nr. 3 BNatSchG), was ihnen einen hohen Schutzstatus im Rahmen des europäischen sowie des deutschen Naturschutzrechts verleiht. Die Vegetation von Zwergstrauchheiden ist an besonders nährstoffarme Bedingungen angepasst. Die Standorte der trockenen Sandheiden sind Podsole oder auch podsolige Böden auf sandigen Sedimenten. Diese Böden sind stark versauert und besonders nährstoffarm. Meist verfügen sie über mächtige Rohhumusauflagen aus schwer zersetzbarer Streu (Scheffer et al. 2002). Die Besenheide (*Calluna vulgaris*, im Folgenden als *Calluna* bezeichnet) ist hier dominant und wird hauptsächlich begleitet von anspruchslosen Moos- und Flechtenarten (Gimingham 1972; Ellenberg 1996). Eine mit der Zeit stattfindende Akkumulation von Nährstoffen ermöglicht es jedoch auch weiteren Arten wie Gräsern und Pioniergehölzen sich zu etablieren und die Dominanz des Zwergstrauches abzulösen. Vergrasung und Verbuschung von Heideflächen sind somit die unweigerliche Folge, sofern der Lauf der Sukzession nicht aufgehalten wird (Heil & Aerts 1993). Heutige Bestände dieser Lebensräume können nur durch geeignete Pflegemaßnahmen im Rahmen eines naturschutzorientierten Flächenmanagements erhalten werden. Pflegemaßnahmen wie Beweidung, Mahd, Plaggen oder Brennen, die ehemals Teil der traditionellen Heidebewirtschaftung waren, zielen neben der Erhaltung des Offenlandcharakters vor allem darauf, dem System Nährstoffe, insbesondere Stickstoff, zu entziehen (Webb 1998; Härdtle et al. 2006). Der Anstieg der N-Depositionsraten jedoch verändert die bisherigen N-Bilanzen des Ökosystems und beschleunigt den natürlichen Akkumulationsprozess von Stickstoff. Die Folgen erhöhter N-Deposition sind somit zu einer zusätzlichen Herausforderung für das Flächenmanagement geworden (Terry et al. 2004; Härdtle et al. 2006).

Die Ergebnisse bisheriger Studien zeigen, dass N-Einträge die Biomassenproduktion von *Calluna* erhöhten (Uren et al. 1997; Lee & Caporn 1998; Carroll et al. 1999). Darüber hinaus war ein Anstieg der N-Konzentrationen in der Biomasse (Lee et al. 1992; Uren et al. 1997; Carroll et al. 1999; Kirkham 2001) sowie im Oberboden feststellbar (Power et al. 1998a;

Pilkington et al. 2005). Im Weiteren war für *Calluna* eine erhöhte Sensibilität gegenüber Stressfaktoren wie Frost oder Dürre sowie verstärkte Herbivorie durch den Heideblattkäfer (*Lochmaea suturalis*) zu beobachten (Berdowski 1993; Power et al. 1998b; Carroll et al. 1999). Als Langzeiteffekt zeigte sich schließlich eine Abnahme der Flechten- und Moosdiversität, eine Zunahme des Auftretens und der Deckung durch Gräser bis hin zu einer großflächigen Vergrasung einstiger Heideflächen (Heil & Diemont 1983; Bobbink 1998; Carroll et al. 1999).

Erhalt und Schutz heute noch vorhandener Heide-Lebensräume erfordern es, Beeinträchtigungen, die durch einen Anstieg der N-Deposition verursacht werden, zu verhindern, zu minimieren oder durch geeignetes Management auszugleichen. Dafür ist ein detailliertes Verständnis der Prozesse erforderlich, welche die bisher aufgezeigten Beeinträchtigungen und Veränderungen bedingen.

Fragestellungen und Methoden

Nährstofflimitierung in trockenen Sandheiden

(Beitrag I: *N:P ratio and the nature of nutrient limitation in Calluna-dominated heathlands* und Beitrag II: *Molinia caerulea responses to N and P fertilisation in a dry heathland ecosystem (NW-Germany)*)

Die großflächige Vergrasung von Heidebeständen gehört zu den Hauptproblemen beim Erhalt und Schutz von Heide-Lebensräumen. Anhaltende N-Depositionen konnten als einer der Hauptgründe für den Artenwechsel identifiziert werden (Heil & Diemont 1983; Bobbink 1998; Carroll et al. 1999). In trockenen Sandheiden wird *Calluna* vor allem durch die Draht-Schmiele (*Deschampsia flexuosa*, im Folgenden als *Deschampsia* bezeichnet) verdrängt. Freiland- und Gewächshausexperimente zeigten, dass *Deschampsia* auch unter erhöhter N-Verfügbarkeit nur in Lücken des geschlossenen *Calluna*-Kronendachs dominant werden kann (Aerts 1993). Die Anfälligkeit *Callunas* gegenüber Stress und Störungen wie Heideblattkäferattacken sowie Frost- und Dürreschäden nimmt jedoch im Zuge anhaltend erhöhter N-Depositionen zu und damit auch die Wahrscheinlichkeit der Lückenbildung durch absterbende *Calluna*-Pflanzen. Somit können hohe N-Depositionen langfristig zur Dominanz von *Deschampsia* führen (Aerts & Bobbink 1999).

Neben der Vergrasung durch *Deschampsia* wird zunehmend auch die Ausbreitung des Gewöhnlichen Pfeifengrases (*Molinia caerulea*, im Folgenden als *Molinia* bezeichnet) in trockenen Sandheiden beobachtet. Während die Ausbreitung der Art in Feuchtheiden seit langem bekannt ist und als relativ gut untersucht gelten kann, sind die Prozesse in trockenen Sandheiden nicht vollständig geklärt (Berendse 1990; Berendse et al. 1994; Taylor et al. 2001; Kaiser & Stubbe 2004). Konkurrenzversuche unter zusätzlicher Nährstoff-Düngung ergaben sich widersprechende Ergebnisse. Bei einem Konkurrenzversuch von Aerts et al. (1990) erwies sich *Calluna* sogar bei einer Düngung von $200 \text{ kg N ha}^{-1} \text{ a}^{-1}$ gegenüber *Molinia* noch als überlegen. Beide Arten profitierten von der Düngung durch Wachstumszunahmen, wobei die Art der Nährstofflimitierung jedoch nicht eindeutig geklärt werden konnte, da hier neben Stickstoff gleichzeitig auch Phosphor und Kalium verabreicht wurde. Bei einem Konkurrenzversuch in Töpfen, durchgeführt von Heil & Bruggink (1987), zeigten sich unter vollfaktorieller N- und P-Düngung bei beiden Arten deutliche Wachstumszunahmen wenn Phosphor verabreicht wurde. *Molinia* konnte die größeren Zuwächse verzeichnen und somit in Konkurrenz mit *Calluna* langfristig an Dominanz gewinnen. Auf N-Gaben hingegen war bei *Molinia* keine Veränderung und bei *Calluna* sogar eine Abnahme der Biomassenproduktion im Vergleich zur Kontrolle (ohne zusätzliche Nährstoffdüngung) festzustellen. Diese

Ergebnisse verdeutlichen, dass die starke Ausbreitung von *Molinia* in *Calluna*-Heiden ein weitaus komplexerer Prozess zu sein scheint als durch bisherige Studien abgebildet und erklärt werden konnte.

Es stellt sich dabei die Frage, inwieweit die Art der Nährstofflimitierung beider Arten zu einem Dominanzwechsel beiträgt (Roem et al. 2002). Ein solcher Einfluss auf die Artenzusammensetzung durch N- oder P-Limitierung konnte zum Beispiel für Kalkmagerrasen festgestellt werden (Aerts & Bobbink 1999). Für Heiden jedoch fehlt es an einem derartigen Nachweis. *Calluna* zeichnet sich durch eine besondere Anpassung an extrem nährstoffarme Standorte aus, wobei ihr Wachstum durch die Verfügbarkeit von Stickstoff limitiert ist (Gimingham 1972). Die Zunahme der N-Verfügbarkeit durch N-Deposition bewirkt somit eine Wachstumszunahme sowie einen Anstieg der N-Konzentrationen in der Biomasse (Lee et al. 1992; Uren et al. 1997; Carroll et al. 1999). Wird der N-Bedarf auf diese Weise ausreichend gedeckt, ist mit einer Verschiebung von N- hin zu einer NP-Kolimitierung und schließlich einer reinen P-Limitierung zu rechnen (Verhoeven et al. 1996; Aerts & Bobbink 1999; Menge & Field 2007). Ein Wechsel von N- zu P-Limitierung könnte dazu führen, dass *Molinia* gegenüber *Calluna* an Konkurrenzvorteilen gewinnen kann (Kirkham 2001). *Molinia* besitzt, als Anpassung an P-limitierte Standorte, arbuskuläre Mykorrhiza, welche die Aufnahme von Phosphor entscheidend verbessern (Fitter 1985; Barus 2003; Javot et al. 2007). *Calluna* hingegen verfügt über eine ericoide Mykorrhizierung, welche die Erschließung organischen Materials und vor allem dessen Nutzung als N-Quelle ermöglicht (Cairney & Burke 1998). Während diese spezielle Art von Mykorrhiza wahrscheinlich entscheidend dazu beiträgt, dass *Calluna* auch bei geringen N-Mineralisationsraten noch existieren kann (Read 1991; Straker 1996), bietet sie jedoch keinen Vorteil bei ausreichender N-Verfügbarkeit. Bei bestehender P-Limitierung sollte *Molinia* somit im Konkurrenzkampf um die Aufnahme von Phosphor Vorteile gegenüber *Calluna* besitzen (Eason et al. 1991; Kirkham 2001; van der Heijden & Sanders 2002).

Zur Feststellung der bestehenden Nährstofflimitierung kann die Biomassenproduktion als Resonanz auf eine faktorielle Düngung von Stickstoff und Phosphor herangezogen werden (Verhoeven et al. 1996; Aerts & Chapin III 2000). Derartige Düngeversuche fanden bisher jedoch selten im Freiland statt. Als weniger zeitaufwändige Methode wird darüber hinaus das N:P-Verhältnis der Biomasse als Indikator für die Nährstofflimitierung von Vegetationsbeständen vorgeschlagen (Koerselman & Meuleman 1996). Ein N:P-Verhältnis kleiner 10 soll nach Güsewell (2004) eine N-Limitierung anzeigen, während ein Wert größer 20 ein Indikator für das Bestehen einer P-Limitierung ist. Bei einem N:P-Verhältnis im Bereich zwischen 10 und 20 kann die Art der Nährstofflimitierung nicht eindeutig bestimmt werden.

Um ein besseres Verständnis für die Hintergründe der zunehmend starken Ausbreitung von *Molinia* in Trocken-Heiden zu entwickeln, sind neben der Art der Nährstofflimitierung auch Informationen über Biomassenallokationsmuster von Interesse. Die mengenmäßige Produktion von blühenden und vegetativen Trieben kann Aufschluss über Wachstumsstrategien und Ausbreitungspotenzial der Grasart geben.

Zur Bestimmung der bestehenden Nährstofflimitierung sowie einer etwaigen Verschiebung der Limitierung von Stickstoff zu Phosphor oder zu einer NP-Kolimitierung wurden Düngeexperimente (N und P, vollfaktorielles Design, n=10) an Freilandbeständen im NSG Lüneburger Heide durchgeführt. Dabei wurden sowohl *Calluna*-dominierte (Beitrag I) als auch *Molinia*-dominierte Bestände (Beitrag II) untersucht. Die Behandlung und Untersuchung fand für *Calluna* in fünf aufeinanderfolgenden Jahren an denselben Beständen statt. Im Falle von *Molinia* handelte es sich um unterschiedliche Bestände in zwei unterschiedlichen Jahren. Ergänzend zu den Freilanduntersuchungen fanden bei beiden Untersuchungen Düngeexperimente gleichen Aufbaus mit einjährigen Pflanzen der jeweiligen Art im Gewächshaus statt, welche für die Dauer einer Vegetationsperiode durchgeführt wurden.

Hauptfragestellung der in Beitrag I und II vorgestellten Untersuchungen:

Ist das Wachstum von *Calluna* bzw. *Molinia* limitiert durch Stickstoff oder Phosphor oder NP-kolimitiert?

Ergänzende Fragestellung bei Beitrag I:

Ist das N:P-Verhältnis der Biomasse von *Calluna* ein geeigneter Indikator zur Beurteilung der vorherrschenden Nährstofflimitierung?

Ergänzende Fragestellung bei Beitrag II:

Zeigen sich für *Molinia* unterschiedliche Biomassenallokationsmuster als Folge der N-Düngung im Hinblick auf die Produktion vegetativer und blühender Triebe?

Einfluss von Stickstoffeinträgen auf die Konkurrenzbeziehung zwischen *Calluna* und *Molinia* während der Pionierphase

(Beitrag III: *Mechanisms of *Molinia caerulea* encroachment in dry heathland ecosystems with chronic nitrogen inputs*)

Neben der Art der Nährstofflimitierung gilt die Annahme, dass weitere Faktoren das Konkurrenzverhältnis zwischen *Calluna* und *Molinia* bestimmen. Studien von Aerts (1990; 1993; 1999) deckten fundamentale Unterschiede zwischen den beiden Arten im Hinblick auf ihren Umgang mit Nährstoffen sowie in Bezug auf ihre Wachstumsstrategien auf.

Calluna zeigt im Vergleich zu *Molinia* einen besonders effizienten Umgang mit Nährstoffen. Aufgenommene Nährstoffe werden zum einen in verholzter Biomasse festgelegt, zum anderen durch Nährstoffrückgewinnung aus absterbenden Blättern weiter genutzt. *Calluna* verfügt zudem über eine vergleichsweise langsame Wachstumsrate, so dass eine erhöhte Nährstoffverfügbarkeit nur langsam in zusätzliche Biomasse umgesetzt werden kann. Der immergrüne Habitus des Zwergstrauches bildet jedoch langfristig ein geschlossenes Kronendach, welches einen wichtigen Vorteil bei der Konkurrenz um Licht darstellt.

Molinia hingegen hat eine geringe Verweilzeit aufgenommener Nährstoffe. Der sommergrüne Habitus erfordert es, dass oberirdische Biomasse zu jeder Wachstumsperiode erneut aufgebaut werden muss, womit der generelle Nährstoffbedarf steigt. Bei steigender N-Verfügbarkeit jedoch kann *Molinia* die zusätzlichen Nährstoffe aufgrund einer schnellen Wachstumsrate schnell in neue Biomasse umsetzen. Dieser Faktor sollte sich bei der Konkurrenz um Nährstoffe und Licht als Vorteil erweisen.

Konkurrenzversuche unter zusätzlichen Düngegaben durchgeführt von Aerts et al. (1990; 1991) zeigen, dass *Calluna* auch bei Düngegaben von $200 \text{ kg N ha}^{-1} \text{ a}^{-1}$ in Konkurrenz mit *Molinia* noch überlegen blieb. Die Autoren führten dies darauf zurück, dass das immergrüne Kronendach geschlossener *Calluna*-Bestände die Etablierung von *Molinia* verhindert. Die Bildung von Lücken im *Calluna*-Bestand wäre demnach eine wichtige Voraussetzung für das Auftreten eines Artenwechsels durch *Molinia*, so wie es auch für *Deschampsia* festgestellt werden konnte (Aerts 1993; Aerts & Bobbink 1999). Diese Theorie wird bestätigt durch Ergebnisse einer Studie von Berendse et al. (1994), im Rahmen derer eine Zunahme der Grasdeckung in durch den Befall von Heidekäfern geschädigten Beständen festgestellt werden konnte. Ebenso beobachtete Bruggink (1993) die Ansiedlung von *Molinia* auf durch Plaggen geöffneten Flächen (beim Plaggen wird die Vegetation sowie der O- und Teile des A-Horizontes entfernt, Niemeyer et al. 2007). Somit käme der Wiederbesiedlung von vegetationslosen Flächen und damit auch der Konkurrenzsituation zwischen *Calluna* und *Molinia* während der Pionierphase (Phase der Wiederbesiedlung, entsprechend der Entwicklungsphasen von *Calluna*-Beständen nach Gimingham 1972) eine besondere Bedeutung im Hinblick auf den Vergrasungsprozess zu. Bisher fehlt es an entsprechenden Studien, die die Konkurrenz während der Pionierphase untersuchen und Aufschluss darüber geben, welchen Einfluss erhöhte N-Deposition sowie die Art der bestehenden Nährstofflimitierung auf deren Ausgang nimmt.

Im Rahmen eines Topfexperimentes wurden die Bedingungen der Pionierphase auf geplagten Flächen simuliert. Dabei wurde das Wachstumsverhalten von einjährigen *Calluna*- und *Molinia*-Pflanzen unter intra- (Monokulturen) und interspezifischer Konkurrenz (*Calluna*-*Molinia*-Mischkulturen) sowie Reaktionsmuster im Hinblick auf N- und P-Gaben (vollfaktorielles Design, $n=10$) untersucht.

Hauptfragestellungen der in Beitrag III vorgestellten Untersuchung:

- 1) Welche Mechanismen (im Hinblick auf Wachstumsstrategien wie Biomassenallokationsmuster und Strategien der Nährstofffestlegung) bestimmen die Konkurrenzfähigkeit von einjährigen *Calluna*- und *Molinia*-Pflanzen in der Pionierphase?
- 2) Wie beeinflusst die Verfügbarkeit von Stickstoff und Phosphor die Konkurrenzfähigkeit der beiden Arten während dieser Phase?

Verbleib von Stickstoffeinträgen in trockenen Sandheiden

(Beitrag IV: *Fate of airborne nitrogen in heathland ecosystems: a ¹⁵N tracer study*)

Die Heiden der Lüneburger Heide haben nachweislich seit mehr als 30 Jahren N-Einträge erhalten (Matzner 1980; Härdtle et al. 2007), die über dem für trockene Sandheiden angenommenen Grenzwert („critical load“) von 10-20 kg N ha⁻¹ a⁻¹ liegen (Bobbink et al. 2002). In diesem Zusammenhang kommt die Frage nach dem Verbleib dieser erheblichen N-Einträge auf. Obwohl die Grundzüge des terrestrischen N-Kreislaufs seit langem bekannt sind (McNeill & Unkovich 2007), gibt es dennoch bisher nur Hinweise auf die vermutlichen N-Senken bestimmter Systeme. Die Fähigkeit einzelner Kompartimente eines Ökosystems, Stickstoff zu binden und damit für eine bestimmte Zeit festzulegen (N-Retentionskapazität) wurde in Trocken-Heiden bisher nur für die organische Auflage näher bestimmt (Kristensen 2001). Informationen über die Verweilzeiten in den einzelnen Kompartimenten (N-Retentionszeiten) fehlen darüber hinaus vollständig.

Die Quantifizierung vorhandener N-Retentionskapazitäten und -zeiten ist nicht nur ein wichtiger Beitrag zum Verständnis der Reaktionen der Pflanzengemeinschaft auf eine erhöhte N-Deposition, sondern kann auch helfen, die Höhe von N-Depositionsraten sowie die im Laufe der Zeit eingetragenen N-Gesamt mengen zum Beispiel anhand von „critical loads“ (Bobbink et al. 2002) oder in Form von Sättigungsgraden (Aber et al. 1998) zu bewerten. Derartige Bewertungsmodelle stellen die Grundlage für politisches Handeln zur Begrenzung von Emissionen dar und sind somit essentiell für die Durchsetzung von Zielen des Naturschutzes (Gauger et al. 2008).

Den Verbleib von N-Einträgen im Hinblick auf eine zeitliche sowie räumliche Dimension nachzuvollziehen ist eine methodisch gesehen nicht einfache Aufgabe. Die Quantifizierung von N-Vorräten einzelner Kompartimente ermöglicht lediglich eine Darstellung grundsätzlicher Verteilungsmuster. Der Vergleich von N-Vorräten zu Beginn und zum Ende von N-Düngeversuchen kann dann Aufschluss geben, in welchen Kompartimenten Stickstoff langfristig akkumuliert wird (Power et al. 1998a; Pilkington et al.

2005). Derartige Ergebnisse sind jedoch von geringer Aussagekraft und geben keine Auskunft über die genauen Verweilzeiten in den einzelnen Kompartimenten.

Wesentlich genaueres Datenmaterial hingegen können ^{15}N -Markierungsversuche liefern. Hierbei wird durch die Applikation des stabilen Isotops ^{15}N der aktuelle N-Eintrag markiert. Die Berechnung der ^{15}N -Wiederfindung für die einzelnen Kompartimente anhand signifikanter Veränderungen der N-Isotopensignatur ermöglicht es dann, den Weg des N-Eintrags innerhalb des Systems quantitativ über einen längeren Zeitraum hinweg zu verfolgen. Die Methode bietet gegenüber klassischen Düngeversuchen den Vorteil, dass die applizierte Menge zusätzlichen Stickstoffs so gering gehalten werden kann, dass Düngeeffekte ausgeschlossen werden können. Zudem ist die Präzision der erzeugten Daten insbesondere für Freilanduntersuchungen auf Ökosystemebene wesentlich höher einzuschätzen (Hauck et al. 1994; Bedard-Haughn et al. 2003; Fry 2006). Während ^{15}N -Markierungsversuche mehrfach erfolgreich für Waldökosysteme eingesetzt werden konnten (Nadelhoffer & Fry 1994; Buchmann et al. 1996; Tietema et al. 1998; Schleppi et al. 1999; Providoli et al. 2006), wurde die Methode in Heiden bisher erst einmalig in einer Feuchtheide eingesetzt (Curtis et al. 2005). Daten für von *Calluna* dominierten Trocken-Heiden fehlen hingegen vollständig.

Der Verbleib von N-Einträgen (^{15}N -Verteilungsmuster innerhalb der Hauptkompartimente, Quantifizierung von ^{15}N -Auswaschungsverlusten) wurde mittels eines ^{15}N -Markierungsversuches für trockene *Calluna*-dominierte Sandheiden im NSG Lüneburger Heide über einen Zeitraum von zwei Jahren ermittelt. Insgesamt 7 Untersuchungsflächen wurden in zwei Teilflächen unterteilt, wobei auf jeweils einer Teilfläche einmalig zu Beginn der Untersuchung ein ^{15}N -Marker appliziert wurde, während die andere als Referenzfläche diente. Über regelmäßige Beprobungen der wichtigsten Kompartimente des Bodens (O-, A- und B-Horizont) sowie der Biomasse (aktuelle *Calluna*-Triebe, ein bis zwei Jahre alte *Calluna*-Triebe, Moosschicht) konnte die Wiederfindung des applizierten ^{15}N -Markers bestimmt werden. Diese diente dann als Grundlage zur Bestimmung von raum-zeitlichen ^{15}N -Verteilungsmustern innerhalb des untersuchten Systems. Zusätzlich wurden ^{15}N -Verluste durch Sickerwasseraustrag ermittelt. Es wurde angenommen, dass sich die untersuchten Heiden in einem fortgeschrittenen Grad der N-Sättigung befinden, was unter anderem durch einen Anstieg des ^{15}N -Austrags über das Sickerwasser zum Ausdruck kommen sollte (Aber et al. 1998).

Fragestellungen der in Beitrag IV vorgestellten Untersuchung:

- 1) Wie hoch ist der Verbleib von N-Einträgen (bis zwei Jahre nach ^{15}N -Applikation) innerhalb des Systems, und welche Kompartimente (Boden, Biomasse) stellen die Hauptsinken dar?

- 2) Gibt es Anzeichen für eine beginnende N-Sättigung?
- 3) Gibt es Anzeichen für Langzeitsenken im Boden, die dem N-Kreislauf Stickstoff nach und nach entziehen können?

Einfluss von N-Deposition und Sommerdürre auf *Molinia caerulea*

(Beitrag V: *Nitrogen deposition increases susceptibility to drought – experimental evidence with the perennial grass *Molinia caerulea**)

Um zukünftige Entwicklungen von Heidelebensräumen prognostizieren und bewerten zu können und damit den Grundstein für ein angepasstes Management zu legen, reicht es nicht aus, einzig die Auswirkungen von N-Depositionen zu berücksichtigen. Klimatische Veränderungen im Rahmen des Klimawandels werden zukünftig ebenfalls Einfluss auf biogeochemische Prozesse sowie auf die Artenzusammensetzung in Heiden nehmen (Llorens et al. 2004; Wessel et al. 2004; Walther 2010). Einflüsse auf das Pflanzenwachstum sind insbesondere durch einen Rückgang der Sommerniederschläge zu erwarten (Shah & Paulsen 2003; Peñuelas et al. 2004; Damgaard et al. 2009), was sich vor allem durch eine Zunahme in der Häufigkeit und Dauer von niederschlagsfreien Zeiträumen (Sommerdürren) äußern wird (IPCC 2007). Für eine realistische Einschätzung von Folgen des Klimawandels ist die Untersuchung von Einzeleffekten jedoch unzureichend. Tatsächlich wirken erhöhte N-Depositionen und Klimawandelfaktoren, zum Beispiel zunehmende Sommerdürren, simultan bzw. Klimawandelfaktoren wirken auf die bereits durch erhöhte N-Depositionen beeinflussten Lebensräume. Bei der Kombination dieser Wirkungsfaktoren können zudem neben den durch erhöhte N-Depositionen oder Sommerdürren erzeugten Einzeleffekten zusätzlich Interaktionseffekte entstehen (Ritchie 2000; Boyer et al. 2003; Barnard et al. 2006). Derartige Interaktionseffekte treten nur unter Kombination zweier Einzeleffekte auf und lassen sich nicht durch die Summe der Wirkungen der beiden Einzeleffekte erklären (Bortz 2005). Für möglichst realistische Prognosen über die zukünftige Entwicklung von Heiden sind somit Studien unerlässlich, welche die Auswirkungen erhöhter N-Depositionen und relevanter Faktoren des Klimawandels einzeln und in Kombination untersuchen (Tylianakis et al. 2008; Baeten et al. 2010).

Bei erhöhter N-Deposition in Kombination mit Sommerdürren ist das Auftreten von Interaktionseffekten wahrscheinlich. Unter simulierten Sommerdürren wurde mehrfach eine Reduktion der Biomassenproduktion festgestellt (Shah & Paulsen 2003; Peñuelas et al. 2004; Damgaard et al. 2009; Baeten et al. 2010). Eine erhöhte N-Verfügbarkeit hingegen führte in mehreren Studien zu einem Anstieg der Biomassenproduktion sowie des Spross-Wurzel-Verhältnisses (Boot 1989; Aerts et al. 1991; Thornton 1991; Ericsson 1995). Eine Zunahme des Spross-Wurzel-Verhältnisses jedoch sollte sich nachteilig auf den

Wasserhaushalt der Pflanzen auswirken, was zu einer Steigerung der Wachstumsreduktion unter Dürre führen sollte (Aerts & Bobbink 1999).

Bei der Simulation von Dürreperioden im Freiland zeigte sich eine Reduktion der Biomassenproduktion oder keine Veränderung des Wachstums bei *Calluna* (Llorens et al. 2004; Peñuelas et al. 2004; Damgaard et al. 2009). Gordon et al. (1999) fanden neben der Abnahme der Biomassenproduktion aufgrund von simulierten Sommerdürren auch einen Anstieg des Anteils abgestorbener Biomasse. Am stärksten war dieser Effekt jedoch, wenn Dürre mit erhöhter N-Verfügbarkeit kombiniert wurde. Dieser Interaktionseffekt traf zwar auf *Calluna*, nicht aber auf den ebenfalls untersuchten Adlerfarn (*Pteridium aquilinum*) zu. Die Ergebnisse der Studie zeigen nicht nur die Bedeutung möglicher Interaktionseffekte, sondern auch, dass die Reaktion gegenüber veränderten Wachstumsbedingungen wie einer erhöhten N-Verfügbarkeit und Dürreereignissen artspezifisch ausfallen kann. Um eine Aussage über den Vergrasungsprozess in trockenen Sandheiden unter den zu erwartenden Klimaveränderungen treffen zu können, ist demnach das Verhaltensmuster von *Calluna* wie auch von konkurrierenden Gräsern entscheidend. Etwaig unterschiedlich geartete Reaktionen der beteiligten Arten sollten Einfluss auf die vorherrschenden Konkurrenzbeziehungen nehmen (MacGillivray et al. 1995; Morecroft et al. 2004) und sind somit entscheidend für die zukünftige Artenzusammensetzung in Heiden. Über das Verhalten von *Molinia* gegenüber Dürre sowie gegenüber Dürre kombiniert mit erhöhter N-Deposition liegen jedoch bisher keine Erkenntnisse vor.

Im Rahmen eines zweijährigen Gewächshausexperimentes wurde das Verhalten von *Molinia* gegenüber Sommerdürre sowie N- und P-Düngung (vollfaktorielles Design, n=10) getestet. *Molinia*-Pflanzen in Töpfen wurden während des ersten Jahres der Untersuchung vorerst nur den Düngebehandlungen unterzogen. Im zweiten Jahr wurde dann die Behandlung mit simulierten Dürreperioden hinzugenommen. Allen Töpfen wurde zudem ein ¹⁵N-Marker verabreicht, um neben Biomassenallokationsmustern ebenfalls Aussagen über N-Allokationsmuster treffen zu können.

Fragestellung der in Beitrag V vorgestellten Studie:

Welches Verhalten zeigt *Molinia*, als ein exemplarischer Vertreter für in trockenen Sandheiden einwandernde Grasarten, gegenüber N-Düngung in Kombination mit simulierten Sommerdürren?

Hypothesen:

- 1) N-Düngung steigert die Biomassenproduktion von *Molinia*, begleitet von einem Anstieg des Spross-Wurzel-Verhältnisses.

- 2) Das Auftreten von Dürreperioden führt zu einer Reduktion der Biomassenproduktion von *Molinia*.
- 3) Dürre in Kombination mit N-Düngung verstärkt den unter Dürre auftretenden Effekt der Wachstumsreduktion.

Ergebnisse und Diskussion

Die trockenen Sandheiden im NSG Lüneburger Heide, die im Mittelpunkt der vorgestellten Untersuchungen standen, waren nachweislich seit mindestens 30 Jahren einer N-Depositionsbelastung von mehr als $20 \text{ kg N ha}^{-1} \text{ a}^{-1}$ ausgesetzt (Matzner 1980; Niemeyer et al. 2005). Aufgrund dieser anhaltend hohen Belastung war davon auszugehen, dass die untersuchten Heiden in fortgeschrittenem Maße beeinträchtigt sein sollten. Zu den zu erwartenden Veränderungen zählte vor allem ein Wechsel von N- zu P-Limitierung oder NP-Kolimitierung. Des Weiteren wurde ein fortgeschrittener Grad der N-Sättigung angenommen, welcher sich durch eine verringerte N-Retentionskapazität des Systems und eine Zunahme der N-Auswaschung ausdrücken sollte.

Im ersten Jahr des Freilandversuches sowie im Gewächshaus zeigte sich die höchste Biomassenproduktion von *Calluna* unter N+P-Düngung (Beitrag I). In den Folgejahren des Freilandversuches jedoch war lediglich ein reiner N-Effekt festzustellen. *Callunas* Triebblängenwachstum war demnach hauptsächlich durch die Verfügbarkeit von Stickstoff limitiert. Der angenommene Limitierungswechsel hin zu einer P-Limitierung oder NP-Kolimitierung hat somit in den untersuchten *Calluna*-Heiden zumindest noch nicht stattgefunden.

Die gefundenen N:P-Verhältnisse in der Biomasse aktueller *Calluna*-Triebe wiesen eine große zeitliche Variabilität auf. Darüber hinaus ließ sich kein Zusammenhang zwischen der Reaktion der N:P-Verhältnisse und dem Triebblängenwachstum gegenüber den experimentellen Nährstoffbehandlungen feststellen. Die Anwendung des N:P-Verhältnisses als Indikator zur Beurteilung der Nährstofflimitierung sollte somit nicht ohne Einschränkung erfolgen. Die Ergebnisse unterstützen demnach die Schlussfolgerungen von Güsewell (2004), wonach klare Aussagen über die Art der Limitierung nur bei einem N:P-Verhältnis niedriger als 10 (N-Limitierung) oder höher als 20 (P-Limitierung) getroffen werden sollten. Zudem ist aufgrund der großen Variabilität der hier festgestellten Werte von differenzierenden Aussagen auf lokaler Ebene abzuraten.

Die oberirdische Biomassenproduktion von *Molinia* im Freiland nahm in 2006 als Folge der P-Düngung, in 2008 (Freiland und Gewächshaus) nach N-Düngung zu (Beitrag II). Der Zuwachs an Biomasse war bei allen drei Telexperimenten begleitet von einer Zunahme der Anzahl blühender Triebe sowie deren Biomasse. Die Biomasse vegetativer Triebe hingegen zeigte nicht bei allen Telexperimenten eine Reaktion auf die Düngebehandlungen. Innerhalb des Gewächshausexperimentes jedoch reagierte sie mit einem sechsfachen Anstieg in Folge der N-Düngung.

Diese Ergebnisse zeigen, dass das Wachstum von *Molinia* vorrangig N-limitiert war. Ausnahme war jedoch die abweichend positive Resonanz auf die P-Düngung im Freiland in 2006. Das Jahr 2006 zeichnete sich im Gegensatz zu 2008 durch eine Dürreperiode im Juli aus. Dieser Unterschied lässt somit darauf schließen, dass die Art der Nährstofflimitierung zusätzlich durch weitere Faktoren beeinflusst werden kann, wie zum Beispiel durch die Wasserverfügbarkeit.

Die festgestellten Reaktionen auf die Düngebehandlungen von *Calluna* und *Molinia* verdeutlichen, dass das Vordringen von *Molinia* in trockenen Sandheiden nicht allein auf eine erhöhte Biomassenproduktion in Folge einer verbesserten N-Versorgung zurückgeht. Die Veränderung der Biomassenallokationsmuster zeigt, dass *Molinia* bei hoher N-Verfügbarkeit besonders in die Produktion von blühenden Trieben investiert. Eine damit verbundene Zunahme der Samenproduktion sollte somit zur Ausbreitung von *Molinia* beitragen und die Wahrscheinlichkeit für eine erfolgreiche Etablierung auf bisher unbesiedelten Heideflächen erhöhen.

Die hier festgestellte N-Limitierung trifft jedoch nicht für alle europäischen Heiden zu. Ein Wechsel von N- zu P-Limitierung konnte mehrfach für Heiden der Niederlande festgestellt werden (Aerts & Berendse 1988; Roem et al. 2002). Es besteht jedoch die Möglichkeit, dass niederländische Heiden bereits einen stärkeren Grad der N-Sättigung erreicht haben, als es für die hier untersuchten Heiden des norddeutschen Tieflandes der Fall war, zumal die durchschnittliche Depositionsbelastung in den Niederlanden doppelt so hoch ausfällt wie in Norddeutschland (Aerts & Bobbink 1999). Dennoch ist auch in Norddeutschland eine zunehmende Vergrasung einstiger Heideflächen zu beobachten, wobei neben *Deschampsia* auch *Molinia* auf trockenen *Calluna*-dominierten Standorten eine starke Ausbreitung zeigt (Kaiser & Stubbe 2004). Zur Klärung dieser Zusammenhänge tragen die Ergebnisse des in Beitrag III vorgestellten Gewächshausversuches bei.

Die Biomassenproduktion von *Calluna* und *Molinia* (Beitrag III) stieg in Folge der N-Düngung. Die Behandlung mit Stickstoff verringerte die unterirdische Biomassenallokation bei *Calluna*, gefolgt von einem Anstieg des Spross-Wurzel-Verhältnisses (Kontrolle: 1,6 vs. N-Behandlung: 4,3). *Molinia* hingegen erhöhte in Folge der N-Düngung seine unterirdische Biomassenallokation (Spross-Wurzel-Verhältnisse von 0,5 bis 1,0). In Mischkulturen nahm *Molinia* 65%, *Calluna* hingegen weniger als 2% des verabreichten Stickstoffs unter N-Düngung auf. Als Konsequenz dieses N-Mangels bei *Calluna* reduzierte sich die Gesamtbiomassenproduktion in Misch- gegenüber Monokulturen um die Hälfte. In Konkurrenz konnte somit nur *Molinia* von den zusätzlichen N-Gaben profitieren. Mit steigender N-Verfügbarkeit sollte demnach das Konkurrenzverhältnis der beiden Arten zunehmend ungleichgewichtiger werden.

Die Ergebnisse zeigen, dass eine verbesserte N-Versorgung maßgeblich dazu beitragen kann, dass *Molinia* gegenüber *Calluna* an Dominanz gewinnen kann. In der Konkurrenz zwischen einjährigen *Calluna*- und *Molinia*-Pflanzen zeigt *Molinia* eine deutliche Überlegenheit aufgrund einer schnellen Wachstumsreaktion und einer hohen Biomassenallokation hin zu den Wurzeln. Beide Eigenschaften begünstigen eine effiziente Nutzung sowie eine hohe Wettbewerbsfähigkeit in Konkurrenz um unterirdische Ressourcen, so dass sich *Molinias* Konkurrenzüberlegenheit bei erhöhter N-Verfügbarkeit noch zusätzlich verstärkt. Konkurrenzversuche von Aerts et al. (1990) haben gezeigt, dass sich *Molinia* gegenüber älteren *Calluna*-Pflanzen auch unter einer N-Düngung von $200 \text{ kg ha}^{-1} \text{ a}^{-1}$ nicht durchsetzen konnte. *Calluna* profitiert im Erwachsenenalter von ihrem immergrünen geschlossenen Kronendach. Bei der Etablierung ist *Molinia* somit auf Lücken im ansonsten geschlossenen *Calluna*-Dach angewiesen (Aerts 1993). Durch intensive Pflegemaßnahmen wie das Plaggen entstehen somit ideale Bedingungen für die Etablierung von *Molinia*. Da Plaggen zugleich jedoch eine wirksame Methode ist, dem System bereits akkumulierten Stickstoff wieder zu entziehen, sollte auf diese Maßnahme im Rahmen eines effektiven Heidemanagements nicht verzichtet werden (Härdtle et al. 2006). Um die Etablierung von *Molinia* auf geplaggtten Flächen dennoch zu verhindern, sollten angrenzende *Molinia*-Bestände während der ersten Jahre nach dem Plaggen regelmäßig zur Blütezeit gemäht werden. Auf diese Weise sollte die Samenausbreitung und damit auch die Besiedlung durch *Molinia* während der Pionierphase minimiert werden können.

Im Rahmen des ^{15}N -Markierungsversuches (Beitrag IV) betrug die ^{15}N -Gesamtwiederfindung 90% im ersten und 76% im zweiten Jahr der Untersuchung. Entgegen der Annahmen war der größte Teil des ^{15}N -Markers in Biomasse oder Boden festgelegt und weniger als 0,05% ging dem System über das Sickerwasser verloren. Die Moosschicht, mit einer ^{15}N -Wiederfindung von 64% im ersten Jahr, stellte die bedeutendste Kurzzeit-Senke des Systems dar, gefolgt von der organischen Auflage des Bodens. Im zweiten Jahr entwickelte sich die Moosschicht von einer Senken- zu einer Quellenfunktion (23% Verlust innerhalb eines Jahres). Dagegen gewann der Boden 11% des applizierten ^{15}N -Markers hinzu und stellte somit im zweiten Jahr die größte Senke dar. Eine geringe ^{15}N -Wiederfindung in den aktuellen Trieben von *Calluna* (< 2%) ließ darauf schließen, dass in der organischen Auflage, wo *Calluna* den Hauptanteil ihrer Feinwurzeln hat, wiedergefundenes ^{15}N nur zu einem geringen Teil für *Calluna* in aufnehmbarer Form zu Verfügung stand.

Somit ist ein hohes Potenzial zur N-Akkumulation des O-Horizontes nicht zwangsläufig auch mit einer hohen Pflanzenverfügbarkeit von Stickstoff verbunden. Da *Calluna* neben leicht verfügbarem Ammonium und Nitrat mittels ericoider Mykorrhiza auch Zugang zu organischen N-Quellen hat (Read 1991; Cairney & Burke 1998), ist anzunehmen, dass neu eingetragener Stickstoff in einer Form immobilisiert wurde, zu der auch mykorrhizierte *Calluna*-Wurzeln

keinen oder zumindest kurzfristig nur einen begrenzten Zugang haben. Dies würde darauf hindeuten, dass der größte Teil des im O-Horizont gefundenen ^{15}N -Markers in mikrobieller Biomasse (Schmidt et al. 1997; Kristensen & McCarty 1999; Zogg et al. 2000) oder als Teil des stabilen N-Pools (Compton & Boone 2002; Currie et al. 2004; Jones et al. 2004) gebunden wurde. Ein hohes N-Immobilisationspotenzial des O-Horizontes erklärt somit eine geringe N-Verfügbarkeit für *Calluna* und damit auch die in Beitrag I festgestellte anhaltende N-Limitierung.

Die Ergebnisse zeigen, dass die untersuchten Heiden trotz anhaltend hoher N-Depositionsbelastung immer noch über eine hohe N-Retentionskapazität verfügen. Diese sowie die geringen ^{15}N -Auswaschungsverluste weisen auf einen geringen Grad der N-Sättigung des Systems hin (Aber et al. 1998). Bestehende N-Retentionskapazitäten sind vor allem dem ausgeprägten N-Immobilisationspotenzial von Podsolböden zuzurechnen (Brady & Weil 2001). Langfristig ist zu erwarten, dass vor allem der B-Horizont des Bodens eine stabile Senke für N-Einträge darstellt. Die N-Immobilisierung des B-Horizontes verhindert nicht nur N-Verluste durch Auswaschung (Nielsen et al. 2000), sondern entzieht eingetragenen Stickstoff gleichzeitig auch dem aktiven N-Pool (Hagedorn et al. 2005).

Wie stark N-Einträge einen Heidelebensraum beeinträchtigen können und welche Folgen dies für die Artenzusammensetzung hat, hängt neben der N-Depositionsrate und -dauer auch vom Alter, den Standortsbedingungen sowie der Nutzungsgeschichte ab. Da für die kurz- bis mittelfristige Akkumulation von N-Einträgen sowohl die Mooschicht, als auch der organische Oberboden eine besondere Bedeutung haben, ist davon auszugehen, dass das N-Retentionsvermögen eines Heide-Ökosystems sowohl vom Vorhandensein als auch von der Zusammensetzung der Vegetationsdecke abhängen sollte, ebenso wie von den Bodenbedingungen. Entscheidende Unterschiede sind in diesem Zusammenhang im Laufe eines Heideentwicklungszyklus zu erwarten, wie der Vergleich mit den Ergebnissen einer simulierten Pionierphase (Beitrag III) zeigt.

Die geringe N-Verfügbarkeit wie sie aus den Ergebnissen des ^{15}N -Markierungsversuches geschlossen werden konnte (Beitrag IV) und durch die anhaltende N-Limitierung bei *Calluna* und *Molinia* (Beitrag I und II) bestätigt wurde, war während der simulierten Pionierphase des Gewächshausversuches (Beitrag III) nicht festzustellen. Die hohen Aufnahmeraten *Molinias* unter N-Düngung weisen auf eine geringe N-Immobilisationskapazität des humusarmen sandigen A-Horizontes. Der Verlust des O-Horizontes in Folge von Plaggen sollte somit einen entscheidenden Einfluss auf das N-Retentionsvermögen sowie auf das N-Immobilisationspotenzial des Bodens für N-Einträge haben. Der auf geplagten Böden eingetragene Stickstoff sollte demnach überwiegend sofort pflanzenverfügbar sein, solange sich noch keine flächendeckende Mooschicht sowie eine organische Auflage ausgebildet haben. Die besonderen Bodenbedingungen der Pionierphase in Kombination mit der

Konkurrenzstärke junger *Molinia*-Pflanzen bilden somit unter erhöhter N-Deposition die notwendigen Rahmenbedingungen für eine langfristige Vergrasung einstiger Heideflächen. Der durchgeführte ¹⁵N-Markierungsversuch deckt mit einem Zeitraum von zwei Jahren lediglich die kurz- bis mittelfristigen Verteilungsmuster des einmalig applizierten ¹⁵N-Markers ab. Weiterführende Untersuchungen im Rahmen dieses Langzeitversuches jedoch lassen weitere wichtige Erkenntnisse über das N-Retentions- und N-Immobilisationsvermögens des Bodens erwarten, die durch die bisherigen Ergebnisse nur angedeutet werden konnten. Darüber hinaus sollten sich anschließende Studien auf einen Vergleich verschiedener Standorte, Entwicklungsphasen oder Pflegemaßnahmen konzentrieren, um ein umfassendes Verständnis entwickeln zu können.

Im Rahmen des in Beitrag V vorgestellten Gewächshausexperimentes verursachte die N-Düngung wie erwartet einen Anstieg der Biomassenproduktion. Die Dürrebehandlung ergab hier entgegen der Erwartung nur einen geringen Rückgang. Trotzdem wurden starke Interaktionseffekte beider Behandlungen gefunden, die sich vor allem durch einen bis zu 10-fachen Anstieg abgestorbener oberirdischer Biomasse zeigte. Es ist anzunehmen, dass die durch N-Düngung verursachte Zunahme der oberirdischen Biomassenproduktion den transpirationsbedingten Wasserbedarf und somit auch die Empfindlichkeit gegenüber Dürre erhöht hat. Eine hohe ¹⁵N-Wiederfindung sowie erhöhte N-Konzentrationen in der abgestorbenen Biomasse deuten zudem darauf hin, dass das Resorptionsvermögen für Stickstoff aus absterbender Biomasse unter Dürre (in Kombination mit N-Düngung) beeinträchtigt war. Die festgestellten Biomassen- und ¹⁵N-Allokationsmuster lassen darauf schließen, dass *Molinia* weder zu einer Anpassung an Dürre, noch zu einer Kompensation der oberirdischen Biomassenverluste - und damit existenziell wichtigen photosynthetisch aktiven Gewebes - in der Lage war. Obwohl *Molinia* eine ausgeprägte Plastizität gegenüber sich verändernden Nährstoffbedingungen (Beitrag III; Aerts et al. 1991) sowie auch beim Verlust oberirdischer Biomasse (Thornton 1991) besitzt, waren keine Anpassungsstrategien an die veränderten Bedingungen durch die Kombination von N-Düngung und Dürre zu beobachten. Es ist somit davon auszugehen, dass ein Anstieg der Häufigkeit von Sommerdürren in bereits durch N-Deposition belasteten Lebensräumen die Konkurrenzfähigkeit von hochproduktiven Arten wie *Molinia* schwächen sollte. Eine Zunahme von Sommerdürren sollte somit auch einen entscheidenden Einfluss auf die *Molinia*-Vergrasung in Heiden haben. Während der Pionierphase sollten Dürreereignisse zudem in besonderem Maße Wirkung zeigen, denn der vegetations- und auflagelose Sandboden trocknet schnell aus, wenn Niederschläge ausbleiben. Da Einjährige im Vergleich zu älteren *Molinia*-Pflanzen außerdem noch keine tiefreichenden Wurzeln besitzen, besteht ein besonderes Risiko, dass die einjährigen Pflanzen Wassermangel erleiden und absterben (Bannister 1964; Mohamed & Gimingham 1970; Diemont 1990; Bruggink 1993). Die

Austrocknungsgefahr für Jungpflanzen besteht jedoch während des Sommers sowohl für *Molinia* als auch für *Calluna*. Dauerbeobachtungen der Keimlingszahl und -entwicklung auf geplagten Flächen von Bruggink (1993) ergaben, dass neue *Calluna*-Keimlinge während der gesamten Vegetationsperiode auftauchten. Eine besonders hohe Zahl jedoch keimte von September bis Oktober. Zu dieser Zeit waren offensichtlich die Keimungsbedingungen aufgrund einer gleichmäßigen Boden- und bodennahen Luftfeuchte vorteilhafter als während des Sommers. Die verspätete Keimung *Callunas* zu einem Zeitpunkt, zu dem auch zukünftig nicht mit anhaltenden Dürreperioden zu rechnen ist, sollte somit ein Konkurrenzvorteil von *Calluna* gegenüber *Molinia* sein, die im Frühjahr und im Sommer keimt.

Ob das gehäufte Auftreten von Sommerdürren jedoch das Potenzial besitzt, die Vergrasung von trockenen Sandheiden durch *Molinia* zukünftig zu verhindern, kann aufgrund der vorliegenden Ergebnisse nicht mit Sicherheit festgestellt werden. Vergleichbare Experimente mit *Calluna* zeigten, dass es zu einer Wachstumsreduktion bei *Calluna* durch Dürre kam (Llorens et al. 2004; Peñuelas et al. 2004; Damgaard et al. 2009). Wurde *Calluna* zusätzlich auch mit Stickstoff gedüngt, zeigte sich eine Verstärkung des Effektes durch Dürre sowie ein erhöhte Sterberate junger Triebe (Gordon et al. 1999). Demnach werden *Calluna* und *Molinia* vor allem durch den in Kombination von N-Düngung und Dürre entstehenden Interaktionseffekt auf die gleiche Weise beeinträchtigt. Eine Veränderung der Konkurrenzsituation ist somit nur zu erwarten, wenn bezüglich der Stärke des Effekts Unterschiede bestehen. Damgaard et al. (2009) stellten fest, dass sowohl *Calluna* als auch *Deschampsia* durch Dürreperioden in ihrem Wachstum beeinträchtigt wurden. Die Beeinträchtigung schien jedoch für *Deschampsia* schwerwiegender zu sein als für *Calluna*, so dass letztere an Deckung zunehmen konnte. Beim Vergleich von Studien zur Wirkung von Dürreperioden ist jedoch anzumerken, dass die dabei festgestellten Ergebnisse durch das jeweilige Untersuchungsdesign (Anzahl der niederschlagsfreien Tage, Zeitpunkt der Dürre während der Vegetationsperiode) sowie besonders im Freiland durch die bestehenden Witterungsbedingungen (Außentemperatur, Bewölkung) beeinflusst werden können. Um Aussagen für die Konkurrenzbeziehung zwischen *Calluna* und *Molinia* treffen zu können, bedarf es somit weiterführender Untersuchungen, die beide Arten im Rahmen einer Untersuchung direkt in ihrer Konkurrenzsituation betrachten.

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N:P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands

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N:P Ratio and the Nature of Nutrient Limitation in *Calluna*-Dominated Heathlands

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ABSTRACT

There is growing evidence from different sources that prolonged high N deposition causes a shift from nitrogen (N) limitation to nitrogen and phosphorus (P) co-limitation or even P limitation in many terrestrial ecosystems. However, the number of ecosystems where the type of limitation has been directly tested by longer-term full-factorial field experiments is very limited. We conducted a 5-year fertilization experiment with N and P in the Lüneburger Heide (NW Germany) to test the hypothesis that, following decades of elevated atmospheric N inputs, plant growth in dry lowland heaths may have shifted from N to N–P co-limitation or P limitation. We also tested whether the plant tissue N:P ratio reflects the type of nutrient limitation in a continental lowland heathland. Experimental plots dominated by *Calluna vulgaris* received regular additions of N (50 kg N ha⁻¹ y⁻¹), P (20 kg P ha⁻¹ y⁻¹), a combination of both, or

water only (control) from 2004 to 2008. Over the whole study period, a highly significant positive N effect on shoot length was found, thus indicating N limitation. We conclude that a clear shift from N limitation to N–P co-limitation or P limitation has not yet occurred. Tissue N:P ratios showed a high temporal variability and no relationship between tissue N:P ratio and the shoot length response of *Calluna* to nutrient addition was found. The N:P tool is thus of limited use at the local scale and within the range of N:P ratio observed in this study, and should only be used as a rough indicator for the prediction of the type of nutrient limitation in lowland heathland on a larger geographical scale with a broader interval of N:P ratio.

Key words: *Calluna vulgaris*; fertilization experiment; nitrogen deposition; nitrogen saturation; plant growth; phosphorus limitation.

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INTRODUCTION

Nitrogen (N) was until recently generally considered to be the principal limiting nutrient for plant growth in many terrestrial ecosystems. However, human activities have dramatically increased the mobility and deposition of reactive forms of N in recent decades (Galloway and others 2004). For

large parts of Europe and North America there is growing evidence that increased N deposition alters the N status of forests, ultimately leading to N saturation (Aber and others 1998; Dise and others 1998; de Schrijver and others 2008). Aber and others (1998) used forests as an example to describe four stages of N saturation. Early stages comprise increased aboveground biomass production, increased foliar N concentrations, and an accumulation of N in the humus layers of the soil. In advanced stages of N saturation, N is leached and aboveground biomass productivity is reduced. From the analyses of two large-scale European databases, de Schrijver and others (2008) deduced that more than 25% of the European forests included in these databases have reached an advanced stage of N saturation as the N losses with seepage water exceeded $5 \text{ kg ha}^{-1} \text{ y}^{-1}$.

In non-forested terrestrial ecosystems under high N deposition, nitrogen dynamics are much less well studied. In heathland and moorland ecosystems dominated by *Calluna vulgaris* (henceforth referred to as *Calluna*) early responses to increased N deposition are similar to those observed in forests and include increased plant growth as well as a build up of nitrogen stores in the aboveground biomass and humus layers (Power and others 1998a; Carroll and others 1999; Cuesta and others 2008). Prolonged exposure to high N inputs results in an acceleration of the life cycle of *Calluna* accompanied by a changing sensitivity to biotic and abiotic stresses (Power and others 1998b). Despite the limited observational or experimental evidence of strongly increased N leaching from heathland and moorland ecosystems (but see Schmidt and others 2004), a model simulation study conducted by Evans and others (2006) showed that the effects of enhanced N deposition on heathland systems are, in the long term, fundamentally comparable to those on forests.

As N saturation advances, it has been hypothesized that plant growth in terrestrial environments becomes (co-)limited by another key element, phosphorus (P) (Verhoeven and Schmitz 1991; Aerts and Chapin 2000; Menge and Field 2007). This shift from N to P limitation may arise because P is required for plant growth in relatively large quantities, and rates of atmospheric P deposition are generally low. In addition, N inputs can decrease the availability and plant uptake of P via effects on mycorrhizae (Gundersen 1998; Turner and others 2003). Generally, the probability of a shift to P limitation resulting from increased N availability should be highest in soils derived from parent material with low P levels, in more acid

soils, and in more weathered soils (Gress and others 2007).

The shift from N to P limitation due to prolonged high N deposition has been inferred from various measures used to determine the type of nutrient limitation. Nutrient addition experiments with a factorial design are generally considered the most straightforward approach to determining nutrient limitation of plant growth (Aerts and Chapin 2000). For example, the results of a 1-year fertilization experiment showed that the growth of *Sphagnum* was N limited at a low-deposition ombrotrophic bog in northern Sweden, whereas it was P limited at a site with moderate deposition in southern Sweden (Aerts and others 1992). The authors concluded that a shift from N to P limitation of the *Sphagnum* layer had occurred because of the recent increase in N deposition. These findings were confirmed by Limpens and others (2004), who observed that P limits *Sphagnum* growth at sites with moderate to high N deposition in the Netherlands and Ireland.

An alternative approach for the analysis of nutrient limitation patterns is to use the nutrient ratios in plant tissues, or ecological stoichiometry (Güsewell 2004). N:P ratios are closely related to the actual nutrient availability, representing a quick and simple alternative to fertilization experiments. N:P ratios have in recent years been used as diagnostic indicators of nitrogen saturation and limitation of vegetative growth by these nutrients (Tessier and Raynal 2003). Bragazza and others (2004) analyzed the N:P ratio in *Sphagnum* plants sampled from ombrotrophic mires in 11 European countries, representing a broad gradient of atmospheric N deposition. The N:P ratios increased steeply at low N deposition, but above a threshold of approximately $10 \text{ kg N ha}^{-1} \text{ y}^{-1}$ a saturating trend was observed. This has been interpreted as a shift from N limited conditions to P (co-)limited conditions.

Koerselman and Meuleman (1996) proposed that critical aboveground biomass N:P ratios could be used to predict N and/or P limitation at the community level. Based on studies of European wetlands, critical N:P ratios were found to be below 14 for N limitation and above 16 for P limitation. However, the generality of these threshold values has frequently been questioned (Tessier and Raynal 2003; Øien 2004; Soudzilovskaia and others 2005). Having reviewed a wide range of field fertilization experiments, Güsewell (2004) proposed that biomass production is N limited at N:P ratios below 10 and P limited at N:P ratios above 20, whereas within this range, the type of nutrient limitation is not unequivocally related to the N:P ratio.

Although there is growing evidence from different sources that chronic N inputs may be causing a shift from N to N–P co-limitation or even P limitation, the generality of this pattern warrants further testing because the number of ecosystems where the type of limitation has been directly tested by longer-term full-factorial experiments in the field is relatively small and alternative approaches (such as tissue N:P ratio) are not unambiguous (Güsewell 2004; Gress and others 2007). Furthermore, the emergence of P limitation will be affected by factors that influence the supply of plant-available P (Gress and others 2007). The most important of these are inherent soil properties, although management regime (Verhoeven and others 1996; Härdtle and others 2009; Akselsson and others 2008) and climatic conditions (Sardans and Peñuelas 2007; van Meeteren and others 2007) also have a major impact on P budgets and availability.

The Lüneburger Heide region in northwest Germany is characterized by nutrient-poor podsolc soils. Due to a long tradition of historical heathland farming and current management measures, dry lowland heaths dominated by *Calluna* are still the predominant vegetation type (Mück 1998; Niemeyer and others 2007). The N deposition rates measured in the Lüneburger Heide are similar to those reported for heathland ecosystems of central Europe and have exceeded the critical loads for dry heathlands for at least 30 years (Matzner 1980; Engel 1988; Härdtle and others 2007). This article describes the results of a 5-year full-factorial field experiment with N and P in the Lüneburger Heide. In this study, we test the hypothesis that growth of *Calluna*-dominated ecosystems in areas with a long history of N deposition in excess of critical loads may have shifted from N to N–P co-limited or even P limited. Furthermore, we examine whether tissue N:P ratios reflect the nature of nutrient limitation on *Calluna* growth. In addition to the field experiment, we conducted a 1-year greenhouse experiment to test the nature of nutrient limitation of *Calluna* seedlings under controlled conditions.

MATERIALS AND METHODS

Study Area

The field study was conducted in the Lüneburger Heide nature reserve (Lower Saxony, NW Germany; 53°15'N, 9°58'E, 105 m a.s.l.), the site with the largest complex of dry heathlands in NW Germany (about 5500 ha). The study area is characterized by Pleistocene sandy deposits. Prevailing soil types are nutrient-poor podzols, with pH (H₂O)

values in the topsoil ranging between 3.0 and 3.5. The climate is of a humid suboceanic type. Mean precipitation is 811 mm y⁻¹ and the mean temperature is 8.4°C (Niemeyer and others 2005). The background deposition in the study area was determined to be 23 kg N ha⁻¹ y⁻¹ and 0.3 kg P ha⁻¹ y⁻¹ (Härdtle and others 2007). The complementary greenhouse experiment took place in greenhouses of the Federal Research Institute for Rural Areas, Forestry and Fisheries (vTI, Hamburg, Germany).

Experimental Design

In the field experiment, a series of 10 replicate blocks was selected in the study area in June 2004. A pilot survey identified suitable areas with *Calluna* cover greater than 95%, *Calluna* stands aged from 10 to 12 years and similar abiotic site conditions. Within these areas, blocks were randomly located on maps. Each of the 10 blocks was divided into four 1.4 m × 1.4 m plots, with a 0.5-m wide buffer strip in between the plots. Each of the four plots per block was assigned to one of four treatments: N, P, N + P, and control. All blocks were fenced to prevent grazing.

In the N plots, 50 kg N ha⁻¹ y⁻¹ was applied as NH₄NO₃. In the P plots, 20 kg P ha⁻¹ y⁻¹ was applied as Na₂HPO₄·2H₂O, and the N + P plots received a combination of both. In the control plots only distilled water was added. Applications started in July 2004; nutrients were dissolved in 2-l distilled water per plot and applied fortnightly using watering cans until October 2004 (that is, on eight occasions). In the following 4 years, treatments began in May and continued through October, with solutions applied on 10 occasions each year. In 2006, the number of blocks was reduced from 10 to 9, because *Calluna* died off in one area during an exceptionally dry summer period.

As the response of individual plant species to nutrient treatments may differ from the overall community response, it is generally necessary to differentiate the nutrient limitation at the species level and at the community level (Aerts and Chapin 2000). However, in the plots analyzed in the present study *Calluna* formed monospecific stands. Thus, responses of *Calluna* to fertilization may be considered representative for the total community response in this system.

For the greenhouse experiment, seeds of 20 different *Calluna* individuals spaced at least 50-m apart (that is, 20 seed families) were collected in the study area in September 2007. Seedlings were raised in germination dishes during winter and, respectively,

16 individuals were planted into pots ($12 \times 12 \times 12 \text{ cm}^3$ in size) at the beginning of May 2008 (using soil material collected from the upper humus horizon in the study area). Ten replicates were used per treatment (that is, N, P, N + P, control), resulting in a total of 40 pots. In the greenhouse experiment, we applied $48 \text{ kg N ha}^{-1} \text{ y}^{-1}$ as NH_4NO_3 , and $4 \text{ kg P ha}^{-1} \text{ y}^{-1}$ as Na_2HPO_4 . N + P treatment pots received a combination of both. In the controls, only distilled water was added. Solutions were applied weekly from May through September.

Sampling and Chemical Analyses

In the field experiment, the length of the current year's shoots of *Calluna* was recorded in October 2004, 2005, 2006, and 2008 as a response variable to fertilization. In each plot, five plants were randomly selected at the start of the growing season. On each of these plants, five stems were chosen at random and tagged with a plastic band. In October, the length increment of five main shoots and the accompanying longest side shoots per stem were measured using a calliper gauge.

On one occasion before (July 2004) and on four occasions after the start of the experiment (October 2004, 2005, 2006, 2008) 20 current year's shoots per plot were collected from the top of randomly selected *Calluna* plants to determine tissue N and P concentrations. Prior to chemical analyses, biomass samples were air dried, ground with a ball mill (Pulverisette 7; Fritsch, Idar-Oberstein, Germany), and re-dried at 105°C before weighing. N contents were analyzed with a C/N-analyzer (Vario EL; Elementar, Hanau, Germany). For P-determination, samples were dissolved in an $\text{HNO}_3\text{-HCl-H}_2\text{O}_2$ solution (Lambert and Hill 1998) and digested using a microwave (MLS-ETHOS; MLS-GmbH, Leutkirch, Germany). Digests were analyzed by means of an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Immediately before the experiment was started in July 2004, three samples (100 cm^3 each) of the soil organic layer and the A horizon were taken in each plot and were analyzed for plant-available N and P to establish whether there were pre-existing differences in nutrient availability prior to the start of treatment additions. The soil samples were thoroughly mixed and stored at -20°C until analysis. Samples were extracted with 0.0125-M CaCl_2 and analyzed immediately for plant-available N (NH_4^+ , NO_3^-) using a spectrophotometer (Spectroquant VEGA 400; Merck, Darmstadt, Germany). For PO_4^{3-} analysis, soil samples were air dried and

sieved ($<2 \text{ mm}$). PO_4^{3-} was extracted from 5-g dry soil by shaking for 90 min in 250-ml double lactate (DL) solution. Samples were filtered, and extracted PO_4^{3-} was measured with an ICP-OES (Hoffmann 1997).

The main part of the soil analyses took place during the second year. In May and October 2005, soil samples were taken and treated as described above, and subsequently analyzed for plant-available N and P, as well as for total C, N, and P. Soil analyses were carried out to quantify the effects of N and P treatments on both plant-available and total N and P pools, and to establish whether current N:P and C:N ratios in the humus layers differ from those recorded at the site 25 years previously. For analysis of NH_4^+ , 10-g fresh soil was shaken for 60 min with 0.0125-M CaCl_2 (Hoffmann 1997). NO_3^- was extracted by shaking 10-g fresh soil for 10 min in 100-ml Aqua bidest (Allen 1989). The samples were filtered and analyzed immediately using an ion chromatograph (IC-DX 120; Dionex, Idstein, Germany). PO_4^{3-} was determined as described above. To examine total C, N, and P concentrations, soil samples were prepared and analyzed in the same way as biomass samples.

In the greenhouse experiment, the aboveground biomass was used to assess response to fertilization. In September 2008, the 16 *Calluna* plants per pot were harvested and weighed after drying for 12 h at 80°C . To determine tissue N and P concentrations, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany), ground with a mixer mill (MM 400, Retsch, Haan, Germany) or cut with scissors (depending on sample size) and re-dried at 105°C before weighing. Total N concentrations were determined as described above. For P-determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550°C for 3 h. The samples were dissolved in an HCl solution (Schlichting and others 1995) and analyzed with an ICP-OES.

Data Analysis

In the field experiment, the effects of N and P addition on shoot increment and tissue N and P concentrations were tested using repeated measures Linear Mixed Models (LMM). Each LMM included the treatment as the fixed effect (N, P, and N \times P interaction), and year and block as random effects (year, block, and year \times block interaction; all as type-III effects). Analyses were undertaken using restricted maximum likelihood (REML) methodology implemented by the Proc Mixed procedure of SAS 9.1 (SAS Institute Inc., Cary, NC). The effects of

N and P addition on soil nutrient concentrations were tested using Generalized Linear Models (GLM). Each GLM included the main effects of block, N and P, and the N \times P interaction.

The N:P ratio may vary considerably within the individuals of a species when these are sampled at different sites (Güsewell 2004). Not only the mean tissue N:P ratios of all plots, but also the N:P ratios of single plots may be important for testing the hypothesis that the tissue N:P ratio is a suitable indicator of the type of nutrient limitation. We, therefore, tested the correlations between tissue N:P ratio of the control plots and the relative change in current year's shoot length of the nutrient treatments, compared to control plots, using Pearson's correlation analysis.

Results of the greenhouse experiment were evaluated by means of one-way ANOVA. To allow for the visualization of changes in aboveground biomass, nutrient concentration and content in a single graph, the approach of Timmer and Stone (1978) was adopted. Aboveground biomass per pot (that is, of 16 *Calluna* plants) was multiplied by tissue nutrient concentration to yield nutrient content of the 16 *Calluna* plants. Trajectories of changes in nutrient concentration and nutrient content after fertilization are plotted in nutrient content—nutrient concentration space. The direction of change allows one to conclude whether increased nutrient content is because a nutrient is limiting (that is, increases in both nutrient concentrations and biomass) or whether it is just due to luxury consumption (increased nutrient accumulation without any gain in biomass). With the exception of the correlation analysis (no transformation), data were log-transformed prior to the analyses. With the exception of the repeated measures LMM all analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

In the field experiment, shoot length response of *Calluna* to fertilization showed a similar pattern from the second to the fifth year, whereas the first year deviated from this pattern (Figure 1). In the first year, shoot length was increased only by adding N + P simultaneously, whereas shoot length was decreased by adding N only. In the following years, there was a positive response in the N and N + P plots. Repeated measures LMM analysis revealed that there was a highly significant N effect on shoot length over the whole study period ($F_{1,111} = 13.3$; $P < 0.001$). No significant P effect ($F_{1,111} = 1.4$; $P > 0.05$) or interaction effect of N

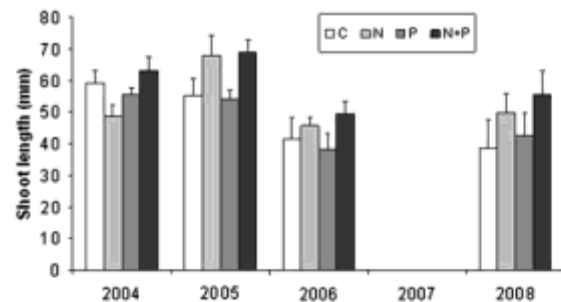


Figure 1. Effects of fertilization on current year's shoot length of *Calluna vulgaris* in the field experiment (2004–2006 and 2008; means and standard errors). C control, N nitrogen, P phosphorus.

and P was found ($F_{1,111} = 2.7$; $P > 0.05$). The mean shoot increment in the control plots was very similar in 2004 and 2005, whereas shoot length was 25% lower in 2006 and 2008 (Figure 1).

In the control plots, N concentrations of current year's shoots increased, and P concentrations decreased throughout the first 3 years of the experiment (Figure 2). As a consequence, the mean tissue N:P ratio increased considerably from 11.1 (2004, range 9–14) to 13.4 (2005, range 10–17) and then 16.9 (2006, range 13–19) (Figure 3). Indeed, in 2006, the vast majority of control plots (8 out of 9 plots) had N:P ratios above 16. In 2008, both the N and the P concentrations showed relatively low values, with an intermediate mean tissue N:P ratio of 13.1 (Figure 2; range 11–17, Figure 3). No significant correlation was found between the N:P ratio of the control plots and the relative change in current year's shoot length of the nutrient treatments, compared to control plots (r between |0.011 and |0.45|; see also Figure 3).

Neither the current year's shoot N and P concentrations nor N:P ratios differed significantly between the treatments before the start of the experiment (data not shown). Tissue N concentrations were significantly increased by the N treatment ($F_{1,111} = 44.4$; $P < 0.0001$) and the interaction between N and P ($F_{1,111} = 4.8$; $P = 0.03$), whereas the P treatment had no significant effect ($F_{1,111} = 2.4$; $P > 0.05$). The P concentrations increased significantly with P addition ($F_{1,111} = 111.5$; $P < 0.0001$), but no N or interaction effect was observed ($F_{1,111} = 0.1$ and $F_{1,111} = 0.02$, respectively; both $P > 0.05$). The addition of N significantly increased the tissue N:P ratio ($F_{1,111} = 5.5$; $P = 0.02$), whereas the addition of P strongly and significantly decreased the tissue N:P ratio ($F_{1,111} = 96.5$; $P < 0.0001$). The N:P ratio

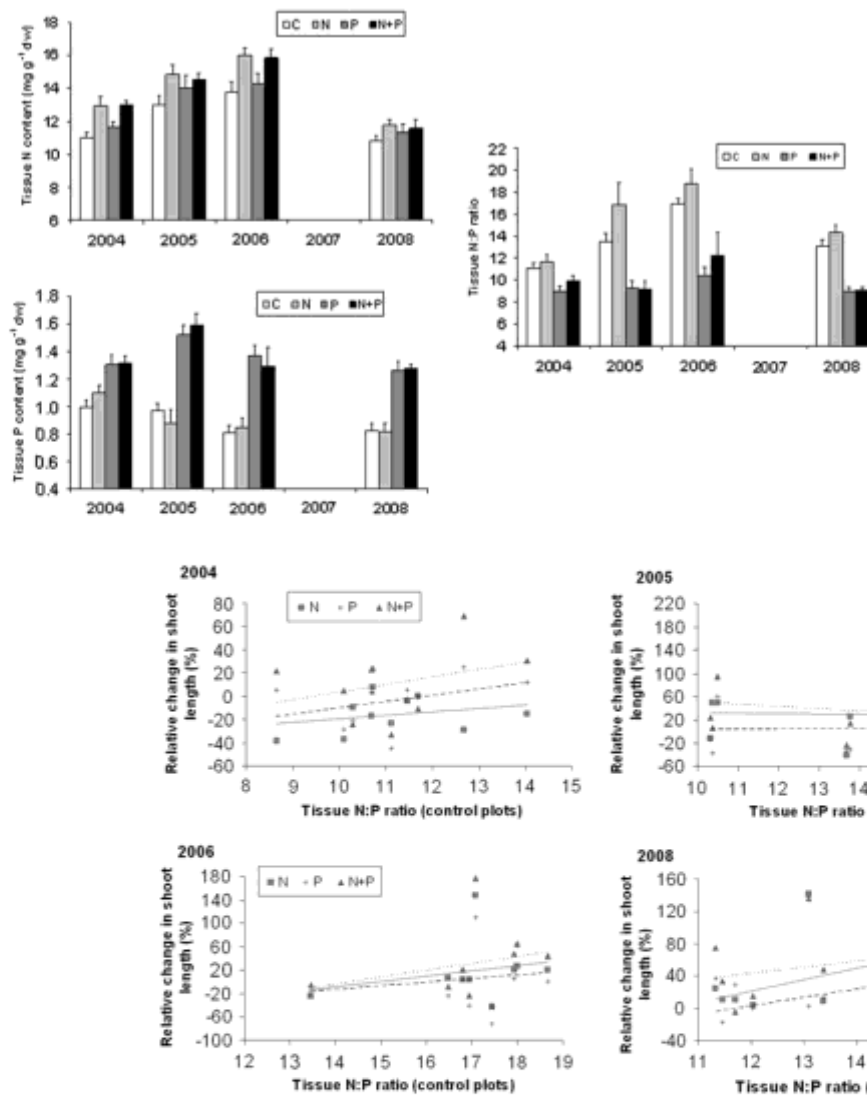


Figure 2. Effects of fertilization on tissue N and P concentrations and N:P ratios of *Calluna vulgaris* in the field experiment [October 2004–2006 and 2008; means and standard errors in mg g⁻¹ dry weight (dw)]. C control, N nitrogen, P phosphorus.

Figure 3. Relationship between tissue N:P ratio of *Calluna vulgaris* (in control plots) and the relative change in current year's shoot length in nutrient treated plots, compared to controls in the field experiment (2004–2006 and 2008): N nitrogen, P phosphorus. Each point represents the mean value of shoot length, measured on five plants per plot (see text). Number of plots in October 2004 and 2005: 10, in October 2006 and 2008: 9. Trend lines: solid line: N treatment; dashed line: P treatment; dotted line: N + P treatment.

was not significantly affected by the N and P interaction ($F_{1,111} = 0.5$; $P > 0.05$).

Soil extractable N and P concentrations were not significantly different between any of the treatments before the start of the experiment (data not shown). The first year's N and P treatments had no significant effect on the NH_4^+ concentrations of the O-horizon at the beginning of the second growing season (May 2005; Table 1). However, significant N

and interaction effects were found for the A-horizon. In October 2005, NH_4^+ concentrations were significantly higher in both soil horizons in N-treated plots. Nitrate concentrations were generally lower than NH_4^+ concentrations and did not differ between treatments. Plant-available PO_4^{3-} was significantly higher in the O-horizon of P-treated plots in May and October 2005. Phosphate concentrations were also highest in the A-horizon of P-

Table 1. Mean Concentrations of Plant-Available NH_4^+ , NO_3^- , PO_4^{3-} , and Total N (N_t), P (P_t) (in mg kg^{-1}) as well as Mean N:P and C:N Ratios in the O-Horizon (O) and A-Horizon (A)

		May 2005					October 2005				
		C	N	P	N + P		C	N	P	N + P	
NH_4^+	O	8.3	9.8	8.8	10.3	$\text{N}^* \text{N} \times \text{P}^{**}$	12.8	17.4	12.7	18.4	N^{**}
	A	0.8	2.1	1.6	1.4		1.4	2.4	1.7	1.9	N^*
NO_3^-	O	0.7	0.8	0.6	0.8	P^*	0.8	1.1	0.9	1.2	
	A	0.8	0.7	0.6	0.7		0.6	0.6	0.6	0.6	
PO_4^{3-}	O	49.9	19.6	116.3	63.2	P^{***}	62.3	49.5	171.0	189.2	P^{***}
	A	6.4	4.5	6.8	6.5		5.9	5.0	7.9	9.5	
N_t	O	11609	10631	14067	10805	$\text{N}^* \text{N} \times \text{P}^*$	15783	16119	13614	14535	P^*
	A	820	1055	970	1086		1085	779	1023	1010	$\text{N}^* \text{N} \times \text{P}^*$
P_t	O	396	339	452	505	P^*	416	614	733	820	P^*
	A	111	89	90	105		96	94	104	90	
N:P	O	24.1	31.3	26.8	21.4	P^{**}	28.0	26.3	17.7	19.5	P^{**}
	A	7.4	11.8	9.1	10.6		11.3	8.3	9.8	12.8	$\text{N} \times \text{P}^*$
C:N	O	24.5	22.8	24.1	23.5	N^*	24.2	23.4	23.8	23.5	
	A	31.6	31.1	33.5	32.2		32.5	33.8	32.7	34.0	

Results of GLM analyses with nitrogen (N), phosphorus (P) and $\text{N} \times \text{P}$ interactions given where significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

treated plots, although the effect was only marginally significant ($P = 0.05$ in May and $P = 0.06$ in October).

Neither total N and P nor the N:P ratios of either horizon varied significantly between treatments in May 2005 (Table 1). In October 2005, however, P fertilization significantly decreased total N in the O-horizon, and the addition of N and N + P resulted in a significant reduction in total N concentrations in the A-horizon. Phosphorus addition significantly increased total P of the O-horizon in October 2005; N:P ratios of this horizon were lowest in the P and N + P plots, reflecting the effect of P treatments. By contrast, in the A-horizon there was a significant effect of N + P treatments on N:P ratios. The C:N ratio of the O-horizon was significantly decreased by N addition in May 2005, whereas no treatment effects were observed for the A-horizon or for either horizon in October 2005.

In the greenhouse experiment, the mean above-ground biomass per pot was significantly higher in the N and N + P treatments than in control and P treatments ($F = 59.7$; $P < 0.001$). Tissue N concentrations were significantly increased in the N and N + P treatment ($F = 120.9$; $P < 0.001$), whereas P addition significantly increased tissue P concentrations ($F = 26.3$; $P < 0.001$). Figure 4A shows that N accumulation was associated with increased aboveground biomass indicating N limitation already in the first year's growth. The response was, however, stronger when N and P were applied simultaneously. The main effect of P addi-

tion was increased P accumulation without gain in aboveground biomass suggesting luxury consumption of the nutrient (Figure 4B).

DISCUSSION

Growth Limitation of *Calluna*

Contrary to our hypothesis, a highly significant N effect on shoot length was found in the field experiment over the whole study period, which is indicative of N limitation. This positive response of *Calluna* to N addition mirrors that seen in some other N addition experiments (Uren and others 1997; Carroll and others 1999). This is also consistent with the results of a short-term N fertilization experiment in the Lüneburger Heide 18 years ago (Mück 1998). In autumn 1989, NH_4NO_3 was added in solid form in a single dose of either 40 or 100 kg ha^{-1} , to pioneer stands of *Calluna*. One year later, shoot length was higher in the fertilized plots (72 and 75 mm, respectively) than in the controls (66 mm). The results of the current study suggest that growth of *Calluna* remains predominantly N-limited, despite a prolonged period of N deposition (Matzner 1980; Engel 1988; Härdtle and others 2007) in excess of the critical load for lowland heathlands (Achermann and Bobbink 2003).

For several reasons, the general conditions in the Lüneburger Heide are suitable for a shift of the heathland community toward N-P co-limitation. (i) In contrast to the high N input rates, P deposi-

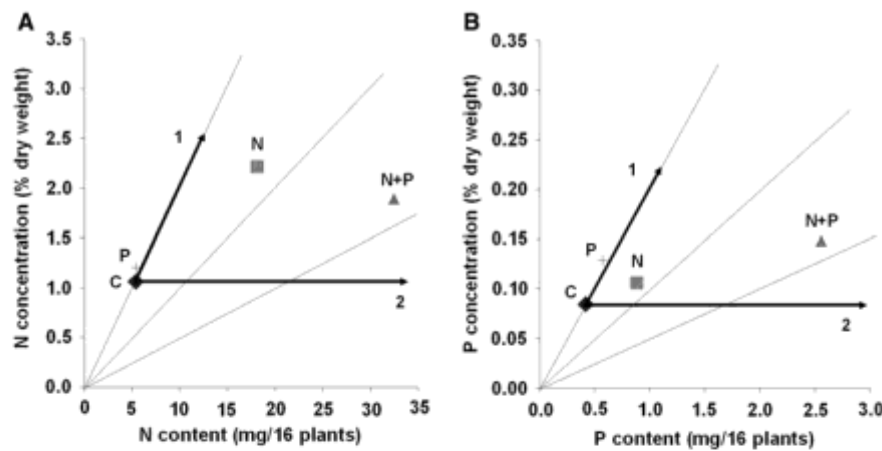


Figure 4. The relationship between tissue nutrient concentration and nutrient content (**A:** N, **B:** P) of the aboveground biomass of 16 *Calluna vulgaris* plants in the greenhouse experiment (data plotted according to the method of Timmer and Stone 1978). Each point represents the mean value of 10 pots. A shift along *arrow 1* denotes increased nutrient accumulation without gain in biomass (luxury consumption), whereas a shift along *arrow 2* indicates increased nutrient content and biomass without change in concentration. A shift into the sector between 1 and 2 denotes increases in both nutrient concentrations and biomass, indicating that the initial level of the nutrient was limiting growth. A shift into the sector below *arrow 2* signifies that the nutrient concentration has been diluted by additional growth.

tion rates have been very low over the last decades (Matzner 1980; Engel 1988; Härdtle and others 2007). (ii) The sandy podzols are a P-poor substrate and have low P fluxes related to mineral weathering, but exhibit high immobilization rates for P (van Meeteren and others 2007). (iii) At all sites where *Calluna* is monodominant, grazing and mowing are applied as regular management measures. Both measures only affect the aboveground biomass, but not the humus layers. Because the aboveground biomass has comparatively high P stores, the overall P budgets of managed heaths are negative (Härdtle and others 2006; Fottner and others 2007).

The question remains in which compartments of a heathland atmospheric N has been accumulated over the years. According to Aber and others (1998) an accumulation of N in the humus layers occurs during early stages of N saturation. In the Lüneburger Heide high-intensity management measures such as sod-cutting were applied only very infrequently during the 1990s. As a result, the thickness of organic layers increased during that time, in line with management plans of the land owner (Verein Naturschutzpark). Concurrently with an accumulation of organic matter in the humus layers, the N:P ratio of the O-horizon has increased from 20.6 to 24.5–31.3 and the C:N ratio decreased from 27.7 to 22.1–24.2 in the Lüneburger Heide during the last 25 years (Matzner 1980; Härdtle and others 2007; Table 1). In addition, leaching rates have in-

creased over the last decades (Matzner 1980; Härdtle and others 2007), and currently about 15% of the total N deposition is lost from the system through seepage water. However, Härdtle and others (2007) found that leaching rates were strongly controlled by internal turnover processes such as mineralization rates within the Lüneburger Heide. An important role in immobilizing atmospherically deposited N may be played by the soil microbial biomass. In a meta-analysis of a wide range of field studies on the effects of N fertilization on microbial biomass Treseder (2008) found an overall decline of microbial biomass under N fertilization. However, Power and others (2006) measured higher microbial biomass and activity in former N-treated plots, 6 years after experimental N additions ceased in a British lowland heath. Nielsen and others (2009) showed that N and P additions had little direct effect on microbial biomass in the soil below a *Calluna* canopy. However, microbial N and P pools were much higher than the pool of inorganic N and P, and the authors concluded that microbes may play an important role in regulating plant nutrient supply. Altogether, the growth response patterns of *Calluna* still indicate N limitation, although the accumulation of N in the humus layers and the N leaching patterns may indicate that the Lüneburger Heide is currently approaching an early stage of N saturation.

In the first year of the field experiment, shoot-length response to fertilization showed a different

pattern than in the following years (Figure 1). Shoot length was negatively affected when only N was added and was positively affected when N and P were applied simultaneously. However, the first year results are not unambiguous, because fertilizer applications started later than in the following years (in July, that is, in the middle of the growing season, when *Calluna* had already achieved 75% of its whole season's growth). In a long-term N addition experiment at an upland moorland in Wales, Carroll and others (1999) found that shoot length of *Calluna* was significantly increased by regular N additions, but not until the second season of the investigation. Carroll and others (1999) attributed the lack of initial response to the late start of N additions, relative to the growing season. In both the field and the greenhouse experiment, N additions considerably increased tissue N concentrations in the first year, indicating that N was readily taken up by *Calluna* plants. Because, significant growth stimulation was observed in the greenhouse experiment in response to N (only) addition, the lack of a growth response in the field experiment is likely to be a reflection of the relatively late-season start of nutrient additions in 2004. However, we cannot explain the positive effect of the combined addition of N and P in the first year of the field experiment.

Enhanced growth associated with elevated rates of N deposition may have increased the standing plant biomass in the Lüneburger Heide during recent decades. However, *Calluna* tissue N concentrations have changed little during this time. End of season (October) foliar concentrations of 13.8 and 13.9 mg N g⁻¹ reported for the late 1970s (Matzner 1980) and mid 1980s (Mück 1998), respectively, are remarkably similar to current concentrations of 10.8–13.7 mg N g⁻¹ (Figure 2; Mohamed and others 2007). N addition experiments conducted by Power and others (1998a) and Pilkington and others (2005) demonstrate the ability of *Calluna* to assimilate N additions for increased growth rates and cover with relatively small increases in tissue N concentration. In our experiment, we observed a significant N treatment effect on tissue N with an increase of about 1–2 mg N g⁻¹ tissue dry weight when compared to the control (Figure 2). However, over long timescales, N concentrations of *Calluna* foliage do not seem to be a good indicator of the ecosystem's N status.

Addition of N and P enhanced plant-available NH₄⁺ and PO₄³⁻ concentrations in the soil in October 2005, which confirms the findings of Nielsen and others (2009). The first year's nutrient additions had no significant effect on the total N

and P concentrations in the beginning of the second year (Table 1). Phosphorus addition in the second year, however, significantly decreased total N in the O-horizon. In this P-poor substrate, P fertilization might increase mineralization rates, therefore releasing more N for plant uptake or leaching and thus reducing total N levels in this horizon. Although not statistically significant, consistent small increases in tissue N concentrations in the P treatments have been found which may be an indication for increased N uptake from the O-horizon (Figure 2).

Interpretation of the results from fertilization experiments must take into account not only the effects of the duration, but also the dose and frequency of N and P additions, sampling dates, and meteorological conditions when evaluating observed plant responses (Aerts and Chapin 2000; Davidson and Howarth 2007). In our field experiment, N additions were twice the annual deposition rates, and P was applied in relatively high doses to take account of high immobilization rates for P in heathland soils (van Meeteren and others 2007). As shoot nutrient contents change during the course of the year, it is important that comparative studies use data from samples collected at the same time of year. The climatic conditions may be the main factor explaining the differences of shoot length in control plots between the study years (Figure 1). In June and July 2006 as well as in May 2008, there were exceptionally dry periods (precipitation was approximately 65% and approximately 80% below the long term average, respectively). Despite its xeromorphic characteristics, *Calluna* is sensitive to spring and summer drought (Gimingham 1960; Gordon and others 1999). As *Calluna* shoot extension takes place predominantly during these months (Gordon and others 1999), the relatively low rainfall during this time is likely to explain the comparatively low *Calluna* shoot lengths in these years (Figure 1).

Tissue N:P Ratio as Indicator of Nutrient Limitation

We found considerable between-year variation in the mean tissue N:P ratios in the control plots (Figure 2). According to the threshold values proposed by Koerselman and Meuleman (1996), this would suggest that the control plots moved from N limitation in the first 2 years to P limitation in 2006 and then back to N limitation in 2008. In ecosystems with highly weathered, acid soils and low P availability in the mineral soil, mineralization through organic matter decomposition is the main

P source for plants and microorganisms (Turner and others 2003). Under desiccated conditions, such as occurred during summer 2006, P mineralization is likely to have been reduced due to decreased soil microbial activity (van Meeteren and others 2007). In addition, P diffusion to the roots is reduced; both these factors result in decreased plant P uptake (Sardans and Peñuelas 2007). However, *Calluna* is adapted to low P availability, and plant P demand may be met partly by P resorption from senescing leaves in this situation (Aerts 1996).

Although a considerable range of tissue N:P ratios was found in the control plots (Figure 3), there was no relationship between foliar N:P ratios and the shoot length response of *Calluna* to nutrient addition in this study. This, together with the high temporal variability in N:P ratios found in both our study and others (Gress and others 2007) strongly implies that the N:P tool should only be used as a rough indicator for the prediction of the type of nutrient limitation in lowland heathland on a larger geographical scale with a broader interval of N:P ratios. Our results reinforce the hypothesis of Güsewell (2004) that the nature of limitation is difficult to predict from N:P ratios unless values are particularly low (<10) or particularly high (>20).

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II *Molinia caerulea* responses to N and P fertilisation in a dry heathland ecosystem (NW Germany)

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Molinia caerulea responses to N and P fertilisation in a dry heathland ecosystem (NW-Germany)

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Abstract In the present study we analysed whether airborne N pollution may constitute one important driver for the encroachment of *Molinia caerulea* in dry heathland ecosystems. Based on full-factorial field experiments (in 2006 and 2008) and complementary greenhouse experiments (in 2008), we quantified growth responses of *Molinia caerulea* to N and P fertilisation (50 kg N ha⁻¹ year⁻¹, 20 kg P ha⁻¹ year⁻¹). Aboveground biomass production of *Molinia caerulea* was limited by P in 2006, but by N in both experiments in 2008. In the greenhouse experiment, N addition caused a sixfold increase of the biomass of vegetative tillers, and in all experiments the biomass and numbers of flowering tillers showed a significant increase due to fertilisation. Our experiments indicated that growth of *Molinia caerulea* was primarily limited by N, but in dry heaths the kind of nutrient limitation may be mediated by other factors such as water availability during the vegetative period. Shifts in biomass allocation patterns resulting from N fertilisation showed that *Molinia caerulea* encroachment in dry heaths is not only attributable to increased leaf biomass, but also due to higher investments in

reproductive tissue that allow for increased seed production and thus accelerated encroachment of seedlings in places where the dwarf shrub canopy has been opened after disturbance.

Keywords Biomass allocation · N:P ratio · Nutrient limitation · Phosphorous supply · Productivity

Introduction

One of the major threats to semi-natural ecosystems and their biodiversity in recent decades has been the increase of airborne nitrogen pollution (Bobbink 1998; van Diggelen and Marrs 2003). Atmospheric N loads affect ecosystem nutrient levels and cycles, the growth of plants and their competition and, ultimately, may cause shifts in plant community composition and biodiversity loss (Stevens et al. 2006). In heathlands, atmospheric N deposition has contributed to an increased aboveground productivity, accelerated nutrient cycles and shifts in the community resilience (van Rheenen et al. 1995; Schmidt et al. 2004; Calvo et al. 2005). Moreover, enhanced N levels are considered one important driver for the replacement of dwarf shrubs by grasses such as *Deschampsia flexuosa* and *Molinia caerulea* (Marrs 1993; Bobbink et al. 1998).

Encroachment of *Molinia caerulea* was initially observed in wet heaths and bogs and was first

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recognised in the Netherlands in the late 1970s/early 1980s, where N deposition was on average 2–8 times higher than in other European countries (Bobbink et al. 1998). Fertilisation experiments aiming at an understanding of the underlying mechanisms showed that stands of *Erica tetralix* are replaced by *Molinia caerulea* swards at high nitrogen levels (Aerts and Bobbink 1999). A transition to *Molinia*-dominated swards occurred when N input rates exceeded values of 17–22 kg ha⁻¹ year⁻¹ (Bobbink et al. 1998). The main factors explaining the vigour of *Molinia caerulea* in wet heaths and bogs at high N levels were (i) a significantly improved productivity, (ii) a high percentage biomass allocation to the roots and (iii) plasticity in the spatial arrangement of leaves over its tall canopy (Aerts et al. 1991).

In dry heathlands, where *Calluna vulgaris* is the main competitor of *Molinia caerulea*, competitive relationships are more complicated, and competition experiments have produced contradictory results (Aerts and Bobbink 1999). In fertilisation experiments of Aerts and Heil (1993) *Calluna vulgaris* was the superior competitor in different nutrient treatments, even at input rates of 200 kg N ha⁻¹ year⁻¹. However, the competitive superiority of *Calluna vulgaris* in this experiment is not in agreement with the experimental results of Heil and Bruggink (1987), nor can it explain the large-scale replacement of *Calluna vulgaris* by *Molinia caerulea* observed in many dry lowland heaths of NW Europe during the last decade (Härdtle et al. 2009). Aerts and Heil (1993) contend that encroachment of *Molinia caerulea* in dry heaths is probably also triggered by stress and disturbance factors, such as senescence, frost, drought, or heather beetle attacks, which weaken the competitive power of *Calluna vulgaris*. Similarly, the replacement of *Calluna vulgaris* by *Deschampsia flexuosa* only occurs when the *Calluna*-canopy is opened as a result of disturbance (Hölzel 2005) or the application of high-intensity management measures (Werger et al. 1985).

Besides the competition experiments mentioned above, *Molinia caerulea* revealed no consistent response pattern to experimental fertilisation with N and P. In some (field and pot) experiments, *Molinia caerulea* growth was limited by N (Thornton 1991; Güsewell et al. 2003; van Heerwaarden et al. 2005), whereas in other cases *Molinia* showed a marked response to P fertilisation (Heil and Bruggink 1987),

or biomass productivity increased significantly only when both N and P were applied (Roem et al. 2002). It is, thus, conceivable that additional factors such as water supply or mycorrhizal infection may mediate experimental outcomes (Aerts and Bobbink 1999). In summary, mechanisms controlling the encroachment of *Molinia caerulea* in dry heaths are not yet well understood, partly due to inconsistencies in experimental results, and partly due to insufficient evidence from experiments analysing growth responses to N or P fertilisation at dry heath sites.

The present study aims at contributing to a better understanding of mechanisms underlying the ongoing process of *Molinia caerulea* encroachment in dry lowland heaths. We hypothesise that growth of *Molinia caerulea* in dry heaths is primarily limited by N, but a shift to P limitation is conceivable as a result of continuing high airborne N loads. Therefore, we analysed growth responses of *Molinia caerulea* to N, P, and N + P fertilisation in a 2-year field experiment and a complementary greenhouse experiment. We focused on growth variables such as biomass of flowering and vegetative tillers as well as the nutritional status of *Molinia caerulea* in order to assess shifts in its competitive performance as affected by fertilisation. The following questions were addressed: (i) Is the aboveground biomass productivity of *Molinia caerulea* limited by N, P or N and P? (ii) Are biomass allocation patterns to flowering and vegetative tillers affected by fertilisation?

Materials and methods

Study area

Field experiments were conducted in the Lüneburger Heide nature reserve (Lower Saxony, NW Germany; 53°15'N, 9°58'E, 105 m a.s.l.), the site with the largest complex of dry heathlands in NW Germany (about 5,500 ha). The study area is characterised by Pleistocene sandy deposits, from which predominantly nutrient-poor podzols developed. The climate is of a humid suboceanic type. Mean precipitation is 811 mm year⁻¹ and the mean temperature is 8.4°C (Niemeyer et al. 2005). The background deposition in the study area was determined to be 23 kg N ha⁻¹ year⁻¹ and less than 0.3 kg P ha⁻¹ year⁻¹ (Härdtle et al. 2007).

Complementary greenhouse experiments took place in greenhouses of the Department of Wood Science (University of Hamburg, Germany).

Experimental design

Field experiment: In March 2006, a first series of 10 replicate plots 10 m × 10 m in size was randomly selected in the study area. Replicate plots were scattered in the nature reserve Lüneburger Heide and thus were spaced apart by at least 100 m (and up to several kilometres). The surface of all replicate plots (i.e. sites where experiment were carried out) was even (i.e. no inclination, no unevenness). *Molinia caerulea* (henceforth referred to as *Molinia*) was mono-dominant (cover 60–90%) in all plots, and ranged in age from 5 to 6 years (according to the management plans of the “Verein Naturschutzpark”). In each of the 10 plots 24 tussocks of *Molinia* were selected by random (distance between tussocks more than 1 m). An area of 0.25 m × 0.25 m surrounding each tussock was defined as subplot and marked with poles. Respectively, 6 subplots per plot were randomly assigned to one of the four treatments: control, N, P, and N + P. All plots were fenced to prevent grazing.

In the N subplots, 50 kg N ha⁻¹ year⁻¹ was applied as NH₄NO₃. In the P subplots, 20 kg P ha⁻¹ year⁻¹ was applied as NaH₂PO₄, and the N + P subplots received a combination of both. In the control subplots, only distilled water was added. Solutions were applied weekly from April through August. The quantities of nutrients applied on each occasion were dissolved in 0.25 l distilled water per subplot and added using watering cans.

In March 2008, a second series of 10 replicate plots was selected and the experiment was repeated in 2008 according to the procedure described above (allowing for a comparison of growth responses of both study years).

Greenhouse experiment: Seeds of *Molinia* of 20 different seed families were collected in the study area in September 2007 (i.e. seeds were collected from individuals spaced apart at least by 50 m). Seedlings were raised in germination dishes during winter and, respectively, 16 individuals planted into pots (12 × 12 × 12 cm³ in size) at the beginning of May

2008 (using soil material collected from the upper humus horizon in the study area). Ten replicates were used per treatment (i.e. N, P, N + P, control), resulting in a total of 40 pots. In the greenhouse experiment we applied 48 kg N ha⁻¹ year⁻¹ as NH₄NO₃, and 4 kg P ha⁻¹ year⁻¹ as NaH₂PO₄ (with regard to the surface of the pots). N + P pots received a combination of both. Fertiliser applications took place in equal time intervals as in the field experiment.

Molinia responses to fertilisation

For the analyses of growth responses to fertilisation we focused on morphological traits that are considered important for the competitive performance of *Molinia* (Tomassen et al. 2003, 2004). Accordingly, we quantified the aboveground biomass production of vegetative tillers (i.e. vegetative tillers with accompanying leaves) and flowering tillers (i.e. tillers with flower stalks and panicles and accompanying leaves). Biomass production was calculated by means of allometries in order to avoid a destructive harvest.

In the field experiment, we determined the number of tillers, the length and width of the leaves as well as the length of culms (of flowering tillers) for each of the 6 tussocks selected per subplot. Numbers of tillers were counted within a ring 20 cm in diameter applied to the tussocks.

In the greenhouse experiment, the same variables were measured for each individual (total *n* of individuals: 640). For all variables means were calculated for each of the 40 pots, based on the measurements of all individuals per pot (i.e. means of 16 individuals).

Measurements were taken when plants were still in a fresh and green status (i.e. at the end of August in 2006/beginning of September in 2008). In order to validate biometric-dry weight relationships 10 tussocks located in the vicinity of the plots were randomly selected, harvested and also analysed for the parameters mentioned above (i.e. biometric data as well as dry weights of vegetative and flowering tillers including weights of leaves and culms). *R*² of the resulting regression model used for the prediction of the biomass dry weight was 0.987 (with *P* < 0.001).

In order to determine tissue N and P concentrations, five vegetative and five flowering tillers of each

of the six tussocks per treatment and plot (in the field experiment) and per pot (in the greenhouse experiment) were pooled and used as a mixed sample (on the same date as biometric measurements were taken). Prior to chemical analyses, samples were air dried, sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany) and re-dried at 105°C before weighing. N contents were analysed with a C/N-analyser (Vario EL; Elementar, Hanau, Germany). For P-determination, samples were dissolved in an $\text{HNO}_3\text{-HCl-H}_2\text{O}_2$ solution (Lambert and Hill 1998) and digested using a microwave (MLS-ETHOS; MLS-GmbH, Leutkirch, Germany). Digests were analysed by means of an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Soil chemical analyses (field experiment)

Soil samples were taken immediately before and after the fertilisation treatments (i.e. in April and August). At each subplot, three soil samples (100 cm³ each) of the upper mineral horizon were taken (sampling depth 5–10 cm), thoroughly mixed and stored at –20°C until analysis. All soil samples were sieved (<2 mm), ground with a mixer mill (MM 400, Retsch, Haan, Germany), and examined for total N and P as well as for plant-available P (PO_4^{3-}) and pH. Total N and P were determined according to the procedure for biomass samples (see above). For the determination of plant-available P, PO_4^{3-} was extracted from 5 g dry soil by shaking for 90 min in 250 ml double lactate (DL) solution (Hoffmann 1997). Samples were filtered, and extracted PO_4^{3-} was measured with an ICP-OES.

Data analysis

In the field experiment treatment effects on biomass production, tissue N and P contents, and soil nutrient concentrations were tested using Generalized Linear Models (GLM). Each GLM included the main effects of block (i.e. plot), N, P and the N × P interaction. Results of the greenhouse experiment were evaluated by means of a one-way ANOVA with post-hoc Tukey's test. Analyses were conducted with SPSS 16.0 (SPSS Inc., Chicago, IL). Data were log-

transformed prior to analyses and the calculation of means and standard errors.

Results

Fertilisation effects on growth of *Molinia*

Field experiment

In 2006, the biomass of both flowering and vegetative tillers increased significantly in the P treatment, whereas neither significant responses to N fertilisation nor N × P interactions were found (Fig. 1a, b; Table 1). Biomass increase in 2006 was due to an increase in both the total number of tillers (i.e. flowering and vegetative tillers; Table 1) and the size of leaves (i.e. leaf length: control 24.0 cm (SE 1.4); N treatment 24.6 cm (SE 1.7), P treatment 24.9 cm (SE 1.3), N + P treatment 25.1 cm (SE 1.4); data on leaf size not shown in Table 1). By contrast, in 2008 the biomass of flowering tillers as well as the number of tillers increased significantly in the N treatment, but no significant effects of the P treatment or of the N × P interactions were found (Fig. 1a, b; Table 1). The biomass increase of flowering tillers only was attributable to an increase in the number of tillers (Table 1), whereas the size of leaves was not significantly affected (leaf length: control 25.5 cm (SE 1.5); N treatment 24.4 cm (SE 1.7), P treatment 23.1 cm (SE 1.5); N + P treatment 25.1 cm (SE 1.5); data on leaf size not shown in Table 1). The biomass of vegetative tillers was not significantly affected by the treatments in 2008.

Greenhouse experiment

In the N and N + P treatment the biomass of vegetative tillers was about sixfold and fivefold higher, respectively, than in the control (leaf length: control 8.5 cm (SE 0.3); N treatment 18.4 cm (SE 0.4), P treatment 8.7 cm (SE 0.2); N + P treatment 19.0 cm (SE 1.0); data on leaf size not shown in Table 1). Flowering tillers were only developed in the N and N + P treatment (Fig. 1c, d). The P treatment had no significant effects on the biomass of both vegetative and flowering tillers.

Fig. 1 Effects of treatments (control, N, P, N + P) on biomass production of vegetative tillers (a and c) and flowering tillers (b and d) of *Molinia caerulea* in the field experiment (2006 and 2008) and the greenhouse experiment, respectively (means + 1SE; biomass data refer to one *Molinia* tussock in the field experiment and to 16 individuals per pot in the greenhouse experiment; in a and b significant differences according to the GLM, see Table 1)

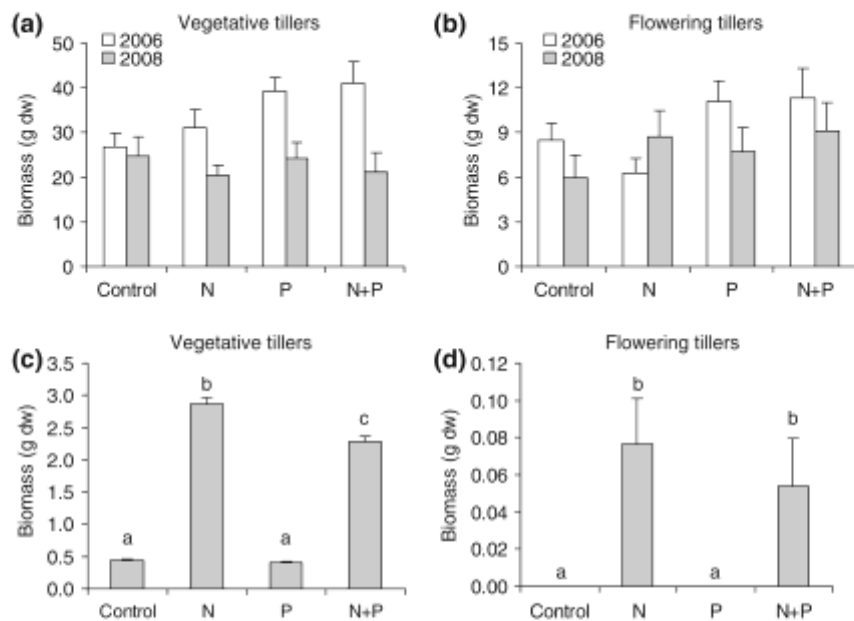


Table 1 Results of the GLM analyses of fertilisation effects on biomass production (flowering and vegetative tillers), number of flowering and vegetative tillers, tissue N and P contents, and tissue N:P ratios of *Molinia caerulea* (field experiment)

Year	Source	df	Biomass of flowering tillers		Biomass of vegetative tillers		Number of flowering tillers		Number of vegetative tillers		Tissue N content		Tissue P content		Tissue N:P ratio	
			F	P	F	P	F	P	F	P	F	P	F	P	F	P
2006	N	1	0.72	0.42	1.21	0.30	0.70	0.43	0.61	0.45	8.58	0.02	1.59	0.24	5.91	0.04
	P	1	16.39	0.003	25.10	0.001	20.24	0.001	55.02	0.000	1.22	0.30	46.51	0.000	68.61	0.000
	N × P	1	0.91	0.37	0.34	0.58	1.59	0.24	0.58	0.47	2.50	0.15	1.95	0.20	0.20	0.66
	Block	9	7.81	0.40	5.75	0.05	2.78	0.30	5.48	0.05	9.73	0.03	8.13	0.03	4.86	0.02
2008	N	1	5.34	0.046	4.46	0.06	5.12	0.049	11.08	0.009	0.12	0.73	2.72	0.13	4.33	0.07
	P	1	0.70	0.42	0.01	0.93	2.87	0.12	0.01	0.91	0.11	0.75	6.41	0.03	38.88	0.000
	N × P	1	0.21	0.66	0.07	0.80	0.60	0.46	0.38	0.56	0.06	0.81	4.29	0.07	7.61	0.02
	Block	9	11.67	0.41	6.74	0.10	15.91	0.25	0.56	0.81	2.42	0.18	4.48	0.01	4.98	0.03

F-ratios and significance values for nitrogen (N), phosphorus (P), the interaction between N and P, and block effects are given (significant results in bold)

df degrees of freedom

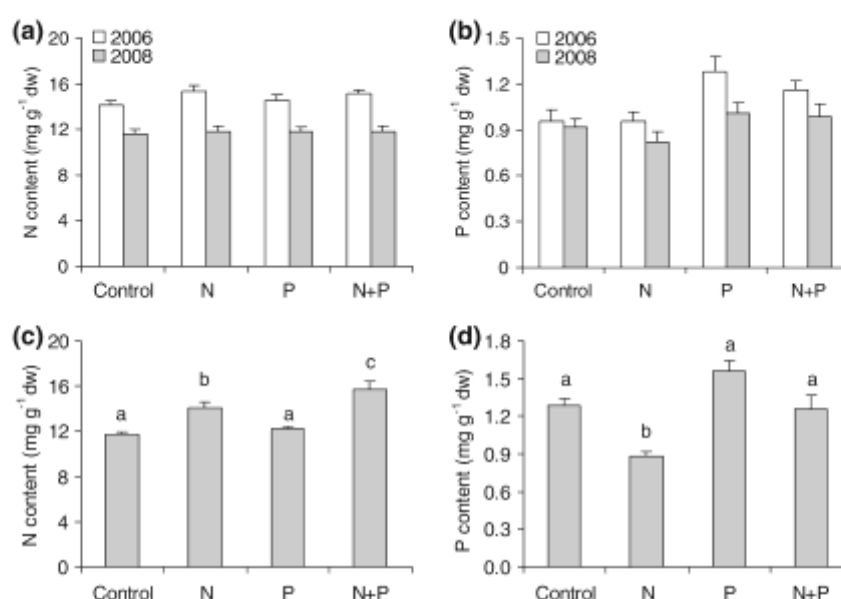
Fertilisation effects on the nutritional status of *Molinia*

Field experiment

In 2006, the N and P contents of the biomass increased due to the N and P treatment, respectively (Fig. 2a, b; Table 1), and both treatments influenced the biomass

N:P ratios (control: 15.2, N treatment: 16.4, P treatment: 11.8; data not shown in Fig. 2). In the control, the biomass N content was 14.1 mg g⁻¹ (vs. 15.4 mg g⁻¹ in the N treatment), and the P content was 0.96 mg g⁻¹ (vs. 1.23 mg g⁻¹ in the P treatment; Fig. 2). In 2008, the N treatment had no effect on the N biomass content and biomass N:P ratios, but the P treatment caused a significant increase in the

Fig. 2 Effects of treatments (control, N, P, N + P) on biomass N (a and c) and P contents (b and d) of *Molinia caerulea* in the field experiment (2006 and 2008) and the greenhouse experiment, respectively (means + 1SE; in a and b significant differences according to the GLM, see Table 1)



biomass P content (control: 0.92 mg g⁻¹; P treatment: 1.01 mg g⁻¹), and a decrease of N:P ratios from 12.7 to 11.8 (control vs. P treatment).

Greenhouse experiment

Biomass N contents were significantly higher in the N and N + P treatment. P addition had no effect on biomass P contents, but influenced the N content negatively. In the N and P treatment biomass N:P ratios were equivalent to 16.0 and 7.8, respectively (control: 9.1; data not shown in Fig. 2).

Fertilisation effects on soil chemical properties (field experiment)

With the exception of pH and N_t (in August 2008), treatments had no significant effects on the soil chemical properties measured (Table 2).

Plant-available PO₄³⁻ ranged between 5.4 and 12.9 mg l⁻¹ (minimum in the control in August 2006), but treatments had no significant affect on the availability of P (in 2006 and 2008). N_t ranged between 1.80 and 2.25 g l⁻¹ in both years and on both sampling dates (i.e. April and August). P_t was between 0.13 and 0.20 g l⁻¹ (both years and both sampling dates; Table 2). The soil N:P ratio was lowest in the control in April in 2008 (10.3) and highest in the N treatment in April in 2008 (16.4; Table 2).

Discussion

Fertilisation effects on growth of *Molinia*

In the greenhouse experiment and the field experiment in 2008 growth of *Molinia* was limited by N. N limitation was particularly evident in the greenhouse experiment, since biomass productivity increased about sixfold and only treatments with N developed generative tillers. However, our hypothesis was not confirmed by the field experiment in 2006. This indicates that additional variables must have affected responses of *Molinia* to fertilisation in the field experiment. We hypothesise that different responses of *Molinia* in the field experiment were attributable to differences in the water availability in the course of the respective vegetation period. The year 2006 was characterised by a severe drought event in July (precipitation of 27 mm in 2006 compared to 178 in 2008), accompanied by elevated July temperatures (mean daily temperature of 21.0°C in 2006 compared to 16.4°C in 2008). According to van Meeteren et al. (2007), microbial P mineralisation, and thus P availability, distinctly decreases when soils start to dry out. This may apply to sandy heath soils in particular, since their water storage capacity is comparatively low (Brady and Weil 2001). It is, therefore, likely that the severe drought in July 2006 worsened the P supply of *Molinia*,

Table 2 Means of soil pH, plant-available PO_4^{3-} (in mg l^{-1}), total N (N_t), total P (P_t ; both in g l^{-1}) and N:P ratios of the upper mineral horizon in the control (C), and treatments (N, P, N + P; +1 SE in brackets; field experiment)

Year	2006					2008					
	Treatment	C	N	P	N + P	Sign	C	N	P	N + P	Sign
pH	April	3.3 (0.03)	3.4 (0.04)	3.4 (0.03)	3.4 (0.03)		3.2 (0.03)	3.2 (0.04)	3.2 (0.03)	3.2 (0.03)	
	August	3.4 (0.03)	3.6 (0.03)	3.6 (0.04)	3.6 (0.03)	**N, ***P	3.5 (0.03)	3.5 (0.02)	3.5 (0.03)	3.4 (0.03)	*N
PO_4^{3-}	April	7.5 (7.2)	11.4 (3.9)	9.7 (1.6)	6.7 (1.6)		11.5 (1.6)	10.9 (1.2)	11.7 (1.6)	10.3 (1.4)	
	August	5.4 (2.7)	12.9 (3.8)	12.9 (2.8)	11.1 (1.5)		9.6 (1.0)	11.0 (1.4)	10.8 (1.4)	11.5 (1.2)	
N_t	April	1.80 (0.22)	2.14 (0.43)	1.96 (0.22)	1.87 (0.19)		2.03 (0.12)	2.09 (0.18)	1.99 (0.18)	2.06 (0.19)	
	August	2.25 (0.45)	2.23 (0.39)	2.16 (0.38)	1.87 (0.23)		1.99 (0.17)	2.05 (0.19)	2.08 (0.21)	2.20 (0.21)	*P
P_t	April	0.14 (0.02)	0.15 (0.03)	0.13 (0.02)	0.13 (0.01)		0.20 (0.01)	0.18 (0.01)	0.18 (0.01)	0.19 (0.01)	
	August	0.15 (0.03)	0.15 (0.03)	0.15 (0.02)	0.14 (0.01)		0.17 (0.01)	0.18 (0.01)	0.18 (0.01)	0.18 (0.01)	
N:P	April	13.3 (1.1)	14.0 (1.2)	14.9 (0.9)	14.0 (1.1)		10.3 (0.5)	16.4 (1.3)	10.8 (0.5)	10.5 (0.7)	
	August	15.2 (0.9)	15.2 (1.2)	15.1 (1.1)	13.7 (1.0)		11.6 (0.7)	11.6 (0.7)	11.6 (0.6)	12.2 (0.7)	

Significant impacts (sign) of the N and P treatment and N × P interactions (according to the GLM analyses) were given (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

resulting in a positive response to P fertilisation. This interpretation is supported by very low values of plant-available PO_4^{3-} found in the control in August 2006 (5.4 mg l^{-1} compared to 9.6 mg l^{-1} found in the control in August 2008).

By contrast, growth of *Molinia* was limited by N in the greenhouse experiment and the field experiment in 2008, probably due to a sufficient water supply *Molinia* experienced in the greenhouse and the humid summer in 2008, respectively. This finding is in agreement with other pot experiments (with sufficient water supply), in which a positive short-term response of *Molinia* to N fertilisation has been demonstrated (deAldana and Berendse 1997; Güsewell et al. 2003; van Heerwaarden et al. 2005). Hence, variable responses of *Molinia* to N and P fertilisation reported from field experiments might be partly due to differences in the water supply *Molinia* experienced in the course of these experiments.

In all experiments *Molinia* responded with an increased biomass (and number) of flowering tillers produced per tussock. This means that fertilisation increases the reproductive performance of *Molinia*, whereas an increase in leaf biomass did not prove to be a consistent response pattern in our experiments. Pot experiments by Thornton (1991) showed that enhanced biomass productivity of *Molinia* through improved nutrition was achieved by an increase in both the number of tillers produced and the size of

various plant parts (such as leaves and basal internodes). In his experiments, high N and high P supply increased the percentage of flowering tillers in particular (from 27% at low N to 60% at high N supply; Thornton 1991), which is in agreement with findings in our field experiment (increase of flowering tillers by 30 and 45% in 2006 and 2008, respectively). In principle, ‘secondary tillering’ as a result of improved nutrient supply seems to be a characteristic growth pattern of *Molinia*, since plants then regularly exhibit a higher number of tillers compared to the total number of buds that were developed at the beginning of the vegetation period (Thornton 1991). As *Molinia* has been proven to shift from an N recycling strategy to reserve formation during growth under high N conditions (van Heerwaarden et al. 2005), plants tend to develop a higher proportion of generative tillers, in which seeds represent important stores for N (Aerts and De Caluwe 1989). Variable response patterns (in terms of a variable number of vegetative tillers) to fertilisation may also be attributable to a trade-off *Molinia* experience with increasing nutrient supply. On the one hand, an increasing biomass allocation to leaves will enhance the competitive performance of plants due to an improved aboveground competitiveness for light. On the other hand, a high aboveground biomass will increase transpiration rates and thus the plant’s water requirements (van Heerwaarden et al. 2005). To meet this

evaporative demand, *Molinia* has to produce a higher number of cord roots that facilitate the transport of water to the aboveground plant parts (Berendse and Aerts 1984; van Heerwaarden et al. 2005). As a consequence, higher biomass allocation to the roots will be required.

In summary, our experiments showed that *Molinia* increased its investment in reproductive tissue under improved nutrient availability. This, in turn, allows for higher seed production and may accelerate the encroachment of seedlings in places where the dwarf shrub canopy has been opened due to management or other disturbance factors (Aerts and Heil 1993). Compared to *Calluna vulgaris*, *Molinia* is the superior competitor during the establishment phase of both species (e.g. after the application of high-intensity management measures), since then (aboveground and belowground) biomass increment rates of *Molinia* by far exceed those of *Calluna vulgaris* under high N availability (Aerts and Bobbink 1999).

Fertilisation effects on the nutritional status of *Molinia*

N treatment increased the tissue N content (greenhouse and field experiment in 2006) and the P treatment the tissue P content of *Molinia* (field experiment), whereas N × P interactions were not found (with the exception of the greenhouse experiment). In addition, both treatments affected tissue N:P ratios. This is in agreement with findings from other experiments (Morton 1977; Berendse et al. 1987a; Tomassen et al. 2004), although a strong increase in aboveground biomass productivity following N fertilisation may also result in unchanged leaf N contents (Tomassen et al. 2004). In the greenhouse experiment, however, N supply of *Molinia* was sufficient despite its high growth rates. The highest concentrations found in the N + P treatment may be attributable to a significantly lower biomass production (compared to the N treatment). In contrast to findings of Berendse et al. (1987b) and Tomassen et al. (2004), pre-senescent biomass P contents and corresponding N:P ratios of *Molinia* were high and low, respectively, in our experiments. This indicates that in our study both tissue nutrient contents and N:P ratios were limited means of predicting *Molinia* responses to N and P fertilisation, particularly as other factors such as drought events might mediate

experimental outcomes. According to Tomassen et al. (2004), aboveground biomass of *Molinia* may be stimulated through N addition even at tissue N:P ratios above 40, which contradicts the finding of Koerselman and Meuleman (1996) that plant growth tends to be limited by P when tissue N:P ratios exceed a threshold of 16. This may also demonstrate the variability of *Molinia* responses to fertilisation in relation to its pre-treatment tissue N:P ratios.

Regarding tissue N and P contents of *Molinia* in the field experiment, we found a significant block effect related to the treatments applied. This indicates that *Molinia* responses to fertilisation (in terms of biomass nutrient contents) were also modified by the variability of site conditions inherent to heathlands and, thus, to our experimental sites. This may be due to differences in soil texture and humus contents, but also to differences in the chemical properties of the experimental sites prior to treatment application (see below).

Fertilisation effects on soil chemical properties

Fertilisation treatments had almost no effects on the soil chemical properties measured (with the exception of soil pH and N_i in August 2008). This may be explained by the principle pathways which nutrients could have taken after addition (i.e. uptake by soil microbes, leaching, soil adsorption, uptake by plants). Based on the results of a long-term N addition experiment in heaths, Power et al. (1998) stated that soil microbes are responsible for the short-term absorption of a high proportion of experimentally added N (see also Johnson et al. 1998). As both total N and P stores of the humus horizons by far exceed the quantities of N and P experimentally added (Härdtle et al. 2006), N_t and P_t (as well as soil N:P ratios) were not significantly affected by the N treatment. In addition, up to 20% of the N added to the system can be leached with seepage water (Härdtle et al. 2007), but leaching rates may vary considerably in relation to the dose and frequency of nutrient additions, sampling dates, and meteorological conditions prevailing during the course of the experiment (Aerts and Chapin 2000).

As regards plant-available PO_4^{3-} , we expected increased values at least in the subplots subjected to P fertilisation. However, the sandy podzols are a P-poor substrate and have low P fluxes related to mineral weathering, but exhibit high immobilisation

rates for P (van Meeteren et al. 2007). This effect, in combination with a rapid uptake of plant-available P by plant roots, may explain why concentrations of plant-available PO_4^{3-} were not significantly affected by the treatments. In 2006, the desiccation of the upper humus layer due to drought might have strengthened the P shortage, at least in the control subplots (van Meeteren et al. 2007).

Conclusions

In summary, high input rates of N may promote *Molinia* encroachment in dry heathland ecosystems, although additional factors such as water availability (in the course of the vegetation period) might mediate *Molinia* responses to improved nutrient availability. In our experiments, *Molinia* showed an increased investment in reproductive tissue resulting from fertilisation. This, in turn, allows for higher seed production and may accelerate the encroachment of *Molinia* seedlings in places where the dwarf shrub canopy has been opened due to management or other disturbance factors. Management of dry heathlands should aim at the removal of flowering tillers of *Molinia* to avoid its encroachment due to the dispersal of seeds. Low-intensity grazing and low-intensity mow might be the best means to counteract the development of generative tillers and thus an ongoing encroachment of *Molinia* resulting from an increasing seed set under high airborne N loads.

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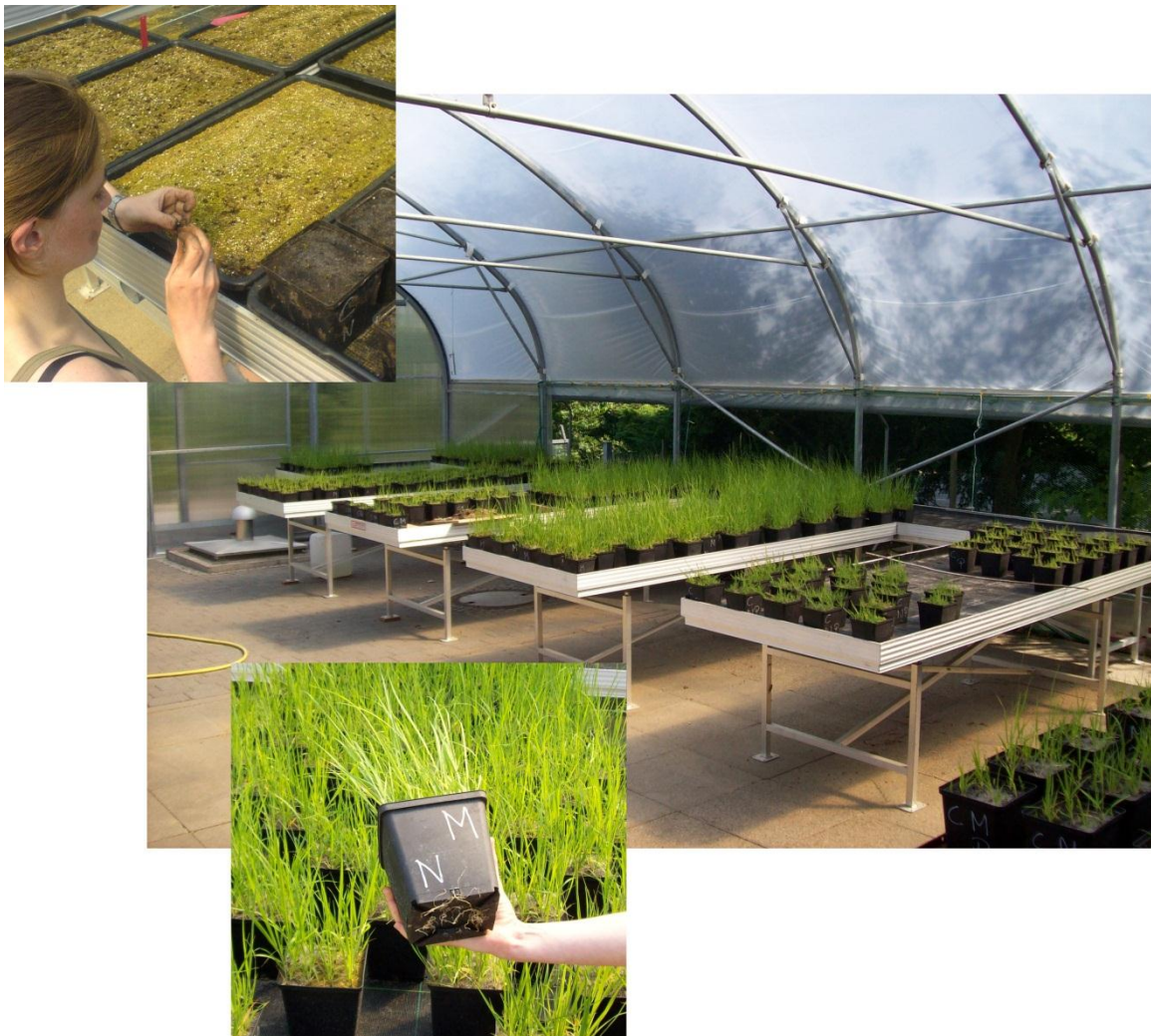
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III

Mechanisms of purple moor-grass (*Molinia caerulea*) encroachment in dry heathland ecosystems with chronic nitrogen inputs

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Mechanisms of purple moor-grass (*Molinia caerulea*) encroachment in dry heathland ecosystems with chronic nitrogen inputs

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Abstract

We analysed growth strategies (biomass allocation, nutrient sequestration and allocation) of heather (*Calluna vulgaris*) and purple moor-grass (*Molinia caerulea*) seedlings in monocultures and mixtures in relation to N, P, and N+P fertilisation in a greenhouse experiment in order to simulate a heath's pioneer phase under high airborne nitrogen (N) loads. N fertilisation increased the total biomass of both species in monocultures. In mixtures, *M. caerulea* sequestered about 65% of the N applied, while *C. vulgaris* suffered from N shortage (halving of the total biomass). Thus, in mixtures only *M. caerulea* will benefit from airborne N loads, and competition will become increasingly asymmetric with increasing N availability. Our results demonstrate that the heath's pioneer phase is the crucial tipping point at which the competitive vigour of *M. caerulea* (high belowground allocation, efficient use of belowground resources, shortened reproductive cycles) induces a shift to dominance of grasses under increased N availability.

Capsule:

The present study indicates that the heath's pioneer phase is the crucial tipping point at which a shift to dominance of purple moor-grass occurs under increased N availability.

Key-words: biomass allocation, *Calluna vulgaris*, competition, N deposition, nutrient sequestration

Introduction

Airborne N loads are a main driver for biodiversity loss worldwide (Sala et al., 2000). Atmospheric N deposition affects ecosystem nutrient levels and cycles, the growth of plants and their competition, and is responsible for shifts in the species composition of many plant communities (Remke et al., 2009; Friedrich et al., 2011). In heathland ecosystems, N deposition has increased primary productivity and N contents of biomass and soil compartments, and contributed to an increasing susceptibility of dwarf shrubs to secondary stress factors such as frost, drought and heather beetle infestation (Uren et al., 1997; Power et al., 1998). In addition, the replacement of dwarf-shrubs by grasses such as wavy hair-grass (*Deschampsia flexuosa*) and purple moor-grass (*Molinia caerulea*) is seen as a long-term effect driven by ongoing N loads (Heil and Diemont, 1983). Due to the dramatic loss of heathland area Europe-wide caused by a far-reaching structural change in agriculture, heaths are nowadays regarded as an internationally endangered habitat type of high conservation value, and conservation efforts are directed towards the preservation of heathland biodiversity (Webb, 1998).

Encroachment of *M. caerulea* was initially observed in wet heaths and bogs, where cross-leaved heath (*Erica tetralix*) is the dominant dwarf shrub. There is evidence that *M. caerulea* gains a competitive advantage after N fertilisation or after lowering the groundwater level (Berendse and Aerts, 1984). Aerts et al. (1991) showed that *M. caerulea* is able to outcompete *E. tetralix* under high N loads because of its improved productivity, its high belowground biomass allocation and its plasticity in the spatial arrangement of leaves.

In dry heathlands, where heather (*Calluna vulgaris*) is the main competitor of *M. caerulea*, competitive relationships are more complicated, and competition experiments have produced contradictory results (Aerts and Bobbink, 1999; Falk et al., 2010).

In fertilisation experiments conducted by Aerts et al. (1990), *C. vulgaris* proved to be the superior competitor, even at fertiliser additions of $200 \text{ kg N ha}^{-1} \text{ y}^{-1}$. Further studies identified fundamental differences between *C. vulgaris* and *M. caerulea* in terms of their nutrient cycling and growth strategies and, thus, their competitive behaviour in dry heaths. *Calluna*

vulgaris, for example, is well adapted to nutrient-poor conditions due to its ericoid mycorrhiza (Read, 1991), its low potential growth rates and a long mean residence time of nutrients in its biomass (Aerts et al., 1991). This “nutrient conserving strategy”, in combination with its evergreen habit, allows *C. vulgaris* to form and sustain a closed canopy all the year round, which in turn may suppress or prevent the establishment of potential competitors, even under increasing nutrient availability (Aerts, 1993a). *Molinia caerulea*, by contrast, is characterised by high potential growth rates, but a short mean residence time of nutrients, which in its turn is accompanied by a higher nutrient demand (Aerts and Berendse, 1988; van Heerwaarden et al., 2005). Because of its deciduous habit, *M. caerulea* has to build up its aboveground biomass at the beginning of each growing season.

If *C. vulgaris* is the superior competitor in dry heaths even under increasing N availability, the question arises as to the nature of the processes underlying the large-scale replacement of *C. vulgaris* by *M. caerulea* observed in many dry lowland heaths of NW Europe during the last two decades (Berendse et al., 1994; Taylor et al., 2001; Falk et al., 2010). Aerts (1993a) contends that *M. caerulea* encroachment in dry heaths occurs when the *C. vulgaris* canopy is opened, for example as a result of senescence, frost, drought, and heather beetle attacks, all of which weaken the competitive power of *C. vulgaris*. This hypothesis is in agreement with the findings of Berendse et al. (1994), according to which the cover of grasses increased after a severe heather beetle attack in NPK-fertilised *C. vulgaris* stands. Bruggink (1993) found that *M. caerulea* seedlings established at dry heath sites where ageing *C. vulgaris* shrubs have been removed by high-intensity management measures such as sod-cutting.

These findings indicate that the dwarf shrub’s rejuvenation phase (i.e. pioneer phase sensu Gimingham, 1972) may be the crucial stage for the establishment of *M. caerulea* in a *C. vulgaris* dominated heath, regardless of whether this has been initiated by natural (e.g. senescence) or man-made disturbance factors (e.g. management). However, studies analysing the competitive relationships between *C. vulgaris* and *M. caerulea* have focused primarily on later successional stages of a heath (e.g. the building or mature phase), and hence paid little attention to the establishment potential of *M. caerulea* in the course of a

heath's pioneer phase. Thus, little is known about the competitive behaviour of *C. vulgaris* and *M. caerulea* seedlings in a heath's pioneer phase, particularly under modifying impacts of airborne N loads. In this context, it is of particular interest to determine whether the species' "growth strategies" (in terms of biomass and nutrient allocation, allometric relationships, and nutrient conserving strategies) that have been analysed with a focus on adult plants also allow a characterisation of the growth behaviour of the species' seedlings. Species-specific growth strategies may change with plant size and thus may be size-dependent (Müller et al., 2000). A plant's growth strategy, however, largely determines its ability to capture resources and to compete with neighbours (Grime, 1979; Tilman, 1988). In a heath's pioneer phase, growth strategies of seedlings of competing species (i.e. *C. vulgaris* vs. *M. caerulea*) may thus determine the course of subsequent successional stages (Gimingham, 1972). *Calluna vulgaris*'s nutrient conserving strategy, for example, should be of minor importance, if seedlings are competing for belowground resources. Adult *M. caerulea* plants, by contrast, are characterised by a high belowground allocation of biomass and nutrients (Aerts et al., 1991). If this also applies to the seedlings, *M. caerulea* should be the superior competitor for belowground resources in a heath's pioneer phase, particularly under elevated N inputs.

The overall objective of the present study is to contribute to a better understanding of the mechanisms underlying the ongoing process of *M. caerulea* encroachment in dry lowland heaths. We hypothesise that the pioneer phase of a heath is the crucial tipping point at which a formerly dwarf shrub dominated heath may become dominated by grasses. We further hypothesise that growth strategies of *C. vulgaris* and *M. caerulea* seedlings are crucial for an understanding of shifts in the species composition (i.e. replacement of *C. vulgaris* by *M. caerulea*), as observed in many European dry lowland heaths.

To this end, we investigated growth strategies of, and competitive relationships between, *C. vulgaris* and *M. caerulea* seedlings in a greenhouse experiment in order to assess their competitive performance by means of a simulated pioneer phase. We focused on growth variables such as biomass and nutrient allocation patterns of both species in monocultures

and mixtures, and analysed shifts in their competitive behaviour as affected by N, P, and N+P fertilisation. The following questions were addressed: (i) What are the main mechanisms (in terms of growth strategies such as biomass allocation and nutrient sequestration) that control the competitive performance of *C. vulgaris* and *M. caerulea* seedlings in a heath's pioneer phase? (ii) How does N and P availability affect the competitive performance of both species in this phase? (iii) Do seedlings and adults of *C. vulgaris* and *M. caerulea* differ in their growth strategies?

Material and methods

Experimental design

Calluna vulgaris and *M. caerulea* seeds were collected in September 2007 in the Lüneburger Heide nature reserve and transferred to germination dishes in a greenhouse without temperature control. Seedlings emerged in the middle of March 2008 (*M. caerulea*) and at the beginning of April 2008 (*C. vulgaris*). For the competition experiment we prepared plant pots (12 x 12 x 12 cm³) that contained nutrient-poor sand (7 cm thick), covered by a humus layer (4 cm thick). The sand was taken from a local sand pit (i.e. C-material typical of podzols; soil ecological characteristics: pH_{H2O}: 7.1, base saturation: 100%, S-value: 4.8 mval 100 g⁻¹, N content: 100 mg kg⁻¹, P content: 95 mg kg⁻¹). The humus material was collected from the upper soil horizons in the Lüneburger Heide nature reserve (soil ecological characteristics: pH_{H2O}: 3.8, base saturation: 21.5%, S-value: 1.7 mval 100 g⁻¹, N content: 800 mg kg⁻¹, P content: 147 mg kg⁻¹). A total of 16 individuals were planted in each pot (4 × 4 rows) in three planting schemes (i.e. competition types): (i) *C. vulgaris* monocultures (i.e. 16 individuals of *C. vulgaris*; henceforth referred to as C-pots); (ii) *M. caerulea* monocultures (i.e. 16 individuals of *M. caerulea*; henceforth referred to as M-pots), and (iii) *M. caerulea*-*C. vulgaris* mixtures (i.e. 8 individuals of *C. vulgaris* and *M. caerulea* respectively; henceforth referred to as CM-pots). This planting scheme was used to mimic natural rejuvenation patterns in a heath's pioneer phase and corresponded to seedling densities characteristic of dry lowland heaths of the Lüneburger Heide nature reserve (Fottner et al., 2004). A total of 40 pots per competition type were set up (i.e. total n of pots was 120). 10 pots per competition type were randomly assigned to four different nutrient treatments: control, N, P, and N+P (i.e. 10 replicates per competition type and nutrient treatment). N-treated pots received 48 kg N ha⁻¹ y⁻¹ (NH₄NO₃), P-treated pots received 4 kg P ha⁻¹ y⁻¹ (Na₂HPO₄) and N+P treated pots received a combination of both. Control pots received deionised water only. Nutrient solutions were applied weekly from the end of May until the end of August. Pots were kept in a greenhouse without temperature control and were watered regularly to

prevent water stress. The pots in the greenhouse were re-arranged randomly every four weeks.

Harvesting and chemical analyses

Plants from all pots were harvested at the beginning of September (when plants were still in a fresh and green state). For the analyses of growth responses to fertilisation we focused on the morphological traits that are considered good indicators of the competitive performance of the respective species (Tomassen et al., 2003; Tomassen et al., 2004). Accordingly, we quantified the aboveground and belowground biomass production of both species in the different competition types and nutrient treatments (by analysing all plant individuals of the pots). In addition, we determined the percentage of flowering plants per pot, the number of tillers per plant (only for *M. caerulea*), the shoot-root ratios, and N and P biomass concentrations (to assess nutrient sequestration and allocation patterns).

After harvest, biomass samples were dried at 80°C for 12 h (until weight constancy) and weighed. Subsequently, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany), ground with a mixer mill (MM 400, Retsch, Haan, Germany), and re-dried over night at 105 °C. N contents were analysed with a C/N analyser (Vario EL cube; Elementar, Hanau, Germany). For P determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550° C for three hours. Ash samples were dissolved in an HCl solution, which was evaporated to dryness. Residues were again dissolved in an HCl solution (Schlichting et al., 1995). Analyses were performed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Data evaluation and statistics

Treatment effects on pot means of biomass dry weights, shoot-root ratios, tissue N and P concentrations and the number of tillers (only *M. caerulea*) were compared by means of one-way ANOVA with a Tukey's post hoc test ($P < 0.05$). Interrelated treatment effects (i.e. type

of fertilisation, competition type) were tested using Generalized Linear Models (GLM). Each GLM included the main effects of N, P, and competition type (henceforth referred to as CT: monoculture vs. mixture) as well as the first order interaction terms (N x P, N x CT, P x CT; see Fig. 1 and Table 1; all data refer to plant individuals of *C. vulgaris* and *M. caerulea*).

In order to evaluate the plants' N and P sequestration, total biomass N and P contents (product of the total biomass and the tissue N and P concentrations) were calculated per pot and species (Fig. 2). To quantify the plants' fertiliser uptake, the total biomass N and P contents of controls were subtracted from the total biomass N and P contents of corresponding treatments. Data in Fig. 2 are presented as percentage uptake from applied quantities of fertiliser per pot and species. One-way ANOVA and Tukey's post hoc test were performed to compare mean fertiliser uptake rates per pot and competition type. Inter- and intraspecific competition was assessed by means of replacement diagrams (Jolliffe, 2000).

All analyses were conducted with SPSS 17.0 (SPSS Inc., Chicago, IL). Non-normally distributed data (percentage of flowering plants per pot) were subjected to Kruskal-Wallis-H-Test with multiple comparisons ($P < 0.05$; Bortz et al., 2008; using STATISTICA 7.1 (StatSoft Inc., Tulsa, OK)).

Results

Treatment effects on C. vulgaris

In the C-pots, aboveground biomass increased by about 60% and 240% as a result of N and N+P fertilisation, respectively (Fig. 1a). Effects of the P treatment were insignificant, but we found a significant N x P interaction on aboveground biomass productivity (Table 1). Belowground biomass decreased as a result of the N treatment (corresponding with a reduction in the relative belowground biomass allocation from 38% to 19%; calculated from data in Table 1), but did not change significantly under N+P fertilisation (Fig. 1b). Again, there was no significant P effect. Shoot-root ratios were highest in the N and N+P treatments due to both a decrease in the belowground (N treatment) and an increase in the aboveground biomass (N and N+P treatments; Fig. 1c). The percentage of flowering plants was only affected in the N+P treatment, and increased from 2% in the control to 48% in N+P treated pots (Fig. 1d).

In the CM-pots, aboveground productivity was distinctly low in all treatments, and productivity was even lower in the N and N+P treatments than in the controls of the C-pots (Fig. 1a). The same response pattern was found for the belowground biomass, and only the N+P treatment enhanced belowground productivity (Fig. 1b). Treatments did not affect the shoot-root ratios in the CM-pots (Fig. 1c). In addition, *C. vulgaris* was almost flowerless in the presence of *M. caerulea* (Fig. 1d).

N and N+P treatments approximately doubled tissue N concentrations of *C. vulgaris* in the C-pots, but effects of these treatments were distinctly reduced in the CM-pots (Table 1). In contrast, P and N+P treatments increased tissue P concentrations in the C-pots. In addition, we found a significant N x P interaction effect on tissue N concentrations.

In the C-pots, *C. vulgaris* sequestered 19% and 43% of experimentally added N in the N and the N+P treatment, respectively (Fig. 2a). P sequestration was 1% in the P and 38% in the N+P treatments. In contrast to the C-pots, N and P uptake of *C. vulgaris* dropped distinctly in the presence of *M. caerulea*, and uptake rates were less than 2% for N and P.

Treatment effects on M. caerulea

In the M-pots, aboveground biomass was more than sixfold higher in the N and fivefold higher in the N+P treatments (Fig. 1e), and N x P interactions on aboveground biomass were significant (Table 1). Belowground biomass quadrupled as a result of N and tripled as a result of N+P fertilisation (Fig. 1f). P treatments affected neither aboveground nor belowground productivity. Both the shoot-root ratios and the number of tillers were twice as high in the N and N+P treatments than in the control pots (Fig. 1g,h), and flowering plants were only developed in the N and N+P treatments (Fig. 1i).

Aboveground and belowground productivity of *M. caerulea* achieved maximum values in the CM-pots subjected to N and N+P fertilisation (Fig. 1e,f), and shoot-root ratios doubled in comparison to controls (Fig. 1g). Similarly, the number of tillers and the percentage of flowering tillers were highest in these treatments (Fig. 1h,i).

N and N+P treatments only slightly affected tissue N concentrations of *M. caerulea* (in both the M- and CM-pots; Table 1). P concentrations were increased as a result of P fertilisation (both competition types), but remained unchanged (M-pots) or decreased (CM-pots) in the N+P treatments. N x P interactions on tissue N and P concentrations were insignificant (Table 1).

In the M-pots *M. caerulea* sequestered 54% (N+P treatment) and 63% (N treatment) of experimentally added N, and uptake rates were hardly affected by the presence of *C. vulgaris* (Fig. 2c). P uptake was highest in N+P treatments (Fig. 2d, both competition types). Calculation of P uptake in P treated CM-pots resulted in a negative value, indicating that P uptake in non-fertilised controls was higher than in P treated pots.

Treatment effects on the competitive performance of C. vulgaris and M. caerulea

Treatment effects on the competitive performance of *C. vulgaris* and *M. caerulea* were assessed by means of replacement diagrams (Fig. 3). Results showed that intraspecific competition was weaker than interspecific competition for *C. vulgaris* (as indicated by a concave curve progression), but was stronger for *M. caerulea* (as indicated by a convex

curve progression). In the N and N+P treatments, the convex shape of the *M. caerulea* curves became more marked (Fig. 3b,d vs. Fig. 3a), indicating an increasing competitive advantage of *M. caerulea* with increasing N supply. In contrast to N and N+P treatments, productivity patterns for both species in the P treatment did not differ from those in the controls (Fig. 3c vs. Fig. 3a).

Discussion

Treatment effects on C. vulgaris monocultures

Responses of *C. vulgaris* to nutrient treatments indicated that growth was NP co-limited, whereby N was the principally limiting nutrient (Table 1, Fig. 1). This was reflected by significant increases in the plants' productivity following N fertilisation, but by maximum biomass values in the N+P treatments (Table 1). In addition, N+P treatments significantly improved the floral development of plants (Fig. 1d). Positive responses of *C. vulgaris* to N fertilisation have also been found in other field or greenhouse experiments (Heil and Bruggink, 1987; Aerts et al., 1991, Uren et al., 1997; Power et al., 1998), although full-factorial additions of N and P have been applied only once in a field study of von Oheimb et al. (2010). A shift from N to P or NP co-limitation may take place when two nutrients (i.e. N and P) are potentially limiting for plant growth, but when fertilisation with N will cause P to become relatively scarce ("resource-ratio hypothesis", Tilman, 1985). In heaths such a shift has been observed as a result of chronic inputs of N from the atmosphere (Verhoeven et al., 1996; Roem et al., 2002). Strikingly, N treatments halved the relative belowground biomass allocation of *C. vulgaris* seedlings (from 38% to 19%), accompanied by a distinct increase of shoot-root ratios (controls: 1.6; N treatment: 4.3; Table 1; indicating a different growth behaviour of seedlings compared to that of the 2-4 year old plants; cf Aerts et al. (1991).

Treatment effects on M. caerulea monocultures

In contrast to *C. vulgaris*, *M. caerulea* was exclusively limited by N. Aboveground and belowground productivity was highest in the N treatment, and N fertilisation doubled the number of tillers per seedling (Table 1). This is in agreement with results found for older plants (Thornton, 1991; Güsewell et al., 2003; van Heerwaarden et al., 2005) according to which *M. caerulea* is characterised by high demands for N, but low P requirements (Roem et al., 2002). In addition, "secondary tillering" as a result of improved N supply seems to be a characteristic growth pattern of *M. caerulea* (Thornton, 1991). In contrast to the study by Heil and Bruggink (1987), *M. caerulea* growth was not promoted in the P treatment, and even

inhibited in the N+P treatment in comparison to the N treatment (Fig. 1e). It is, thus, likely that additional factors such as water supply or mycorrhizal infection may mediate outcomes of fertilisation experiments (Aerts and Bobbink, 1999; Falk et al., 2010). Decreasing growth responses in the N+P treatment may be attributable to ion competition between NO_3^- and PO_4^{3-} , resulting in a decreasing N uptake in the N+P fertilised pots (Fitter and Hay, 2002). As indicated by low shoot-root ratios (ranging between 0.5 and 1.0), *M. caerulea* seedlings showed a high percentage biomass allocation to the roots, even in the N treatment. Seedlings thus exhibit allocation patterns that are well comparable to older plants (shoot-root ratios of 2-4 year old *M. caerulea* showed the same range in the study by Aerts et al. (1991)). These findings have important implications for the sequestration of nutrients and, thus, the competitive performance of *M. caerulea* seedlings (see discussion in the following paragraph).

Treatment effects on competitive interactions between C. vulgaris and M. caerulea

In the CM-pots *M. caerulea* was clearly the superior competitor to *C. vulgaris*. This was attributable to the fact that *M. caerulea* seedlings have the potential to utilise an improved N availability to multiply their biomass production. N fertilisation, for example, increased total biomass of *C. vulgaris* seedlings by a factor of 1.2, but total biomass of *M. caerulea* seedlings by 4.8 (Table 1). This positive response pattern of *M. caerulea* to increasing N availability was particularly pronounced in the CM-pots, expressed by a ninefold increase in the total biomass of *M. caerulea* seedlings (in comparison to the control M-pots), accompanied by a halving of the total biomass of *C. vulgaris* seedlings (in comparison to the control C-pots). *Molinia caerulea* seedlings thus exhibited a much stronger response to N fertilisation than that known from older plants (cf. Aerts et al., 1991)

Based on our findings, we hypothesise that the competitive superiority of *M. caerulea* over *C. vulgaris* is linked to the following (partly interrelated) mechanisms, all of which will favour *M. caerulea* encroachment in a heath's pioneer phase under high N loads:

(i) The high belowground biomass allocation of *M. caerulea* seedlings; (ii) A decisive advantage of *M. caerulea* seedlings in the competition for belowground resources (i.e. N); (iii) A (subsequently) high aboveground productivity; (iv) Shortened reproductive cycles of *M. caerulea* (i.e. after one year) in high N environments; (v) NP co-limitation of *C. vulgaris* seedlings (instead of a sole N limitation of *M. caerulea* seedlings); (vi) A reduced relative belowground allocation of *C. vulgaris* seedlings following N fertilisation.

(i) *Molinia caerulea* seedlings showed a distinctly higher belowground biomass allocation than *C. vulgaris* seedlings. This was mirrored by the very low shoot-root ratios found for *M. caerulea* (0.5 and 1.0 in the controls and N treatments of the M-pots, respectively; Table 1). *Calluna vulgaris*, in contrast, achieved shoot-root ratios of 1.6 and 4.3 (controls and N treatments of C-pots, respectively). As a consequence of a high belowground investment, *M. caerulea* seedlings suppressed the root development of *C. vulgaris* seedlings in all treatments in the CM-pots, as reflected by a reduced *C. vulgaris* belowground biomass and unchanged shoot-root ratios for *M. caerulea*.

(ii) The high belowground biomass allocation of *M. caerulea* seedlings explains their competitive superiority for belowground resources (Goldberg, 1990). An impressive finding was that N sequestration of *M. caerulea* seedlings was unaffected by the presence of *C. vulgaris*, since N uptake rates of *M. caerulea* in the N treatment were almost the same in the M- and CM-pots (about 65%; Fig. 2c). N sequestration of *C. vulgaris*, in contrast, showed a distinct decrease as a result of interspecific belowground competition (Fig. 2a), although *C. vulgaris* may partly compensate a lower belowground allocation by a higher specific root length as well as by its ericoid mycorrhizas (Boot, 1989; Read, 1991, Aerts, 1993b). In our experiments, the high N uptake of *M. caerulea* hardly affected the plants' tissue N concentrations (Table 1). This is indicative of the high N demands of *M. caerulea* seedlings and the use of sequestered N to increase the plants' biomass production (Aerts and Berendse, 1988; van Heerwaarden et al., 2005). By contrast, low tissue N concentrations of *C. vulgaris* seedlings (in the CM-pots) showed that growth was limited by N in the entire course of the experiment. This interpretation is supported by the finding that productivity of *C.*

vulgaris in mixtures did not differ for N and N+P treatments, because the plants' N demand was not satisfied in competition with *M. caerulea* (Fig. 1a,b).

For our experiment we rule out that mycorrhizal infection rates of plant roots in the pots significantly differed from those under field conditions, which in turn might have affected experimental outcomes. There is evidence from both pot and field experiments that mycorrhizal infection takes place in parallel with the development of the fine roots of the plants (immediately after their development; Fyson and Oaks, 1992; Smith, 2009). Since the infection rate of roots follows a logistic growth curve, high infection rates are achieved within a few weeks after seedling growth (Fyson and Oaks, 1992; Smith, 2009).

(iii) As a consequence of the superior competition for belowground resources, *M. caerulea* seedlings multiplied their aboveground productivity in the N and N+P treated pots. Seedlings thus responded even more strongly to N fertilisation than older plants (cf. experiments by Aerts et al., 1991). As shown in Fig. 3, N fertilisation strengthened the intraspecific competition among *M. caerulea* seedlings, and their biomass almost doubled in the CM-pots as a result of reduced intraspecific competition (compared to the M-pots under N fertilisation; Table 1). *Molinia caerulea* seedlings clearly optimise their productivity (and their investment in reproductive tissue; see following paragraph) by an optimal exploitation of plant-available N resources, and *C. vulgaris* seedlings were unable to interfere with this process. Although *M. caerulea* seedlings proved to be strong competitors to *C. vulgaris* seedlings even in unfertilised pots, our results demonstrated that only *M. caerulea* seedlings will benefit from increasing N availability. Thus, competition will become increasingly asymmetric with increasing N inputs. In contrast, growth strategies that are typical of many *Ericaceae* adapted to low N environments (such as low potential growth rates, production of long-living tissue), prove to be a competitive disadvantage when N availability increases (Aerts, 1990; Aerts et al., 1991; Aerts, 1993b). The nutrient-conserving strategy and the evergreen habit of *C. vulgaris* may explain its competitive vigour in closed stands, for example in the late building phase of a heath when *C. vulgaris* suppresses *M. caerulea* encroachment even under high N

loads (Aerts, 1993a). In the pioneer phase of a heath, however, when seedlings compete for belowground resources, these traits are less effective, particularly in high N environments.

(iv) *Molinia caerulea* was capable of shortening its reproductive cycle to one year, as indicated by a high percentage of fertile plants in the N treatments (Fig. 1i). Since *M. caerulea* may also shift from an N recycling to a reserve formation strategy under high N conditions (van Heerwaarden et al., 2005), plants tend to develop a higher proportion of generative tillers (Aerts and de Caluwe, 1989; Falk et al., 2010). Both strategies increase the number of seedlings in the pioneer phase and hence accelerate *M. caerulea* encroachment in places where the dwarf shrub canopy has been opened (Aerts and Heil, 1993).

(v) *Calluna vulgaris* seedlings proved to be NP co-limited, while *M. caerulea* seedlings were exclusively limited by N. Since current deposition rates are low for P, but high for N (Härdtle et al., 2006), they disproportionately support the competitive vigour of *M. caerulea* plants. This applies to both the biomass increment rates and the investment in reproductive tissue (i.e. seed formation).

(vi) In contrast to growth strategies that are known from older plants (cf. Aerts et al., 1991), *C. vulgaris* seedlings halved their relative belowground allocation as a result of increasing N availability. This growth behaviour (resulting in shoot-root ratios > 4) may increase the seedlings' susceptibility to drought events, and thus mortality rates during periods of drought (Power et al., 1998). High N inputs may already have weakened the rejuvenation of *C. vulgaris* in the last decades by this mechanism. Since European heaths will face both high inputs of N and shifting climatic conditions in the near future (Galloway et al., 2004; IPCC, 2007), increasing seedling mortality may contribute to a deterioration of *C. vulgaris*'s rejuvenation success in European heathlands.

Conclusions

Our study demonstrated that the pioneer phase of a heath is the crucial tipping point in a heath's succession, in which *M. caerulea* establishment may induce a transition from a formerly dwarf shrub dominated heath to grass dominated swards. Important traits for the

competitive superiority of *M. caerulea* seedlings in the pioneer phase of a heath are high biomass allocation to the roots, an efficient use of belowground resources (in terms of high N sequestration) and, hence, high aboveground productivity (which in turn improves the plants' competitive vigour for light). In addition, N loads shorten the plants' reproductive cycle, accelerating seed production, which then starts in the pioneer phase of a heath. *Calluna vulgaris* seedlings, by contrast, may suffer from the combined effects of high N loads and increasing drought events due to their low belowground allocation (and thus high shoot-root ratios) in high N environments.

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Table 1. Means and standard errors (in parentheses) of aboveground, belowground and total biomass (in mg dryweight (dw)), shoot-root ratios, tissue N and P concentrations (in mg g⁻¹ dw) per plant individual of *C. vulgaris* and *M. caerulea*, and number of tillers (only *M. caerulea*) under the different fertilisation treatments (C: control; N: nitrogen; P: phosphorus; N+P: nitrogen and phosphorus) in monocultures and in mixtures. Results of GLM analyses with interactions of nitrogen (N), phosphorus (P) and competition type (CT; i.e. monoculture vs. mixture) are given where significant: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

<i>C. vulgaris</i>	<i>C. vulgaris</i> monocultures				<i>C. vulgaris</i> - <i>M. caerulea</i> mixtures				Interactions
	C	N	P	N+P	C	N	P	N+P	
Biomass	32.5	52.2	28.0	109.7	8.9	16.1	10.2	17.7	N x P**
above (mg)	(3.1)	(4.7)	(1.6)	(8.2)	(0.8)	(0.9)	(0.6)	(2.1)	N x CT**
Biomass	20.2	12.4	18.8	28.1	4.7	7.1	5.6	8.3	N x P**
below (mg)	(1.4)	(1.1)	(0.9)	(2.8)	(0.4)	(0.8)	(0.7)	(1.3)	N x CT**
Biomass	52.7	64.6	46.8	137.9	13.6	23.2	15.9	26.0	N x P**
total (mg)	(4.3)	(5.5)	(2.3)	(10.4)	(1.1)	(1.7)	(1.2)	(3.4)	
Shoot-root	1.6	4.3	1.5	4.1	2.0	2.4	2.1	2.2	N x CT***
ratio	(0.1)	(0.3)	(0.1)	(0.3)	(0.2)	(0.2)	(0.3)	(0.1)	
N _{conc}	10.0	20.8	10.6	17.7	12.0	13.8	13.5	13.0	N x P***
(mg g ⁻¹)	(0.2)	(0.6)	(0.2)	(0.7)	(0.3)	(0.5)	(0.3)	(0.3)	N x CT*** P x CT*
P _{conc}	1.0	1.0	1.2	1.4	1.6	1.2	1.7	1.3	N x CT***
(mg g ⁻¹)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	P x CT***
<i>M. caerulea</i>	<i>M. caerulea</i> monocultures				<i>C. vulgaris</i> - <i>M. caerulea</i> mixtures				Interactions
	C	N	P	N+P	C	N	P	N+P	
Biomass	27.4	177.1	24.9	145.6	70.8	334.4	59.1	284.6	N x P***
above (mg)	(1.2)	(4.5)	(1.2)	(6.0)	(4.9)	(8.9)	(4.0)	(12.3)	N x CT***
Biomass	50.7	190.6	43.3	143.8	142.0	334.2	107.6	288.1	N x CT**
below (mg)	(2.6)	(11.2)	(3.3)	(10.1)	(11.1)	(15.0)	(9.0)	(13.8)	
Biomass	78.1	374.2	68.2	289.4	212.8	668.5	166.7	572.7	
total (mg)	(3.6)	(14.8)	(4.5)	(15.5)	(15.3)	(18.0)	(12.8)	(20.5)	
Shoot-root	0.5	1.0	0.6	1.0	0.5	1.0	0.6	1.0	
ratio	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	
Tillers	1.5	3.1	1.4	3.0	2.4	4.0	2.2	3.9	
	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	
N _{conc}	6.4	8.6	7.1	9.9	6.3	10.3	7.1	9.6	P x CT*
(mg g ⁻¹)	(0.2)	(0.2)	(0.2)	(0.4)	(0.2)	(0.4)	(0.2)	(0.2)	
P _{conc}	1.0	0.6	1.7	1.0	1.4	0.7	1.6	0.8	P x CT***
(mg g ⁻¹)	(0.1)	(0.0)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.0)	

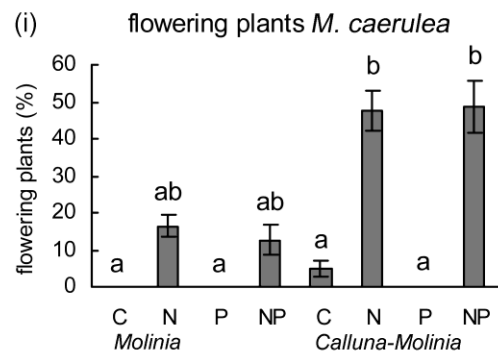
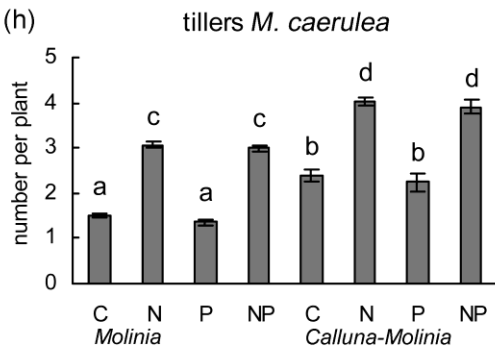
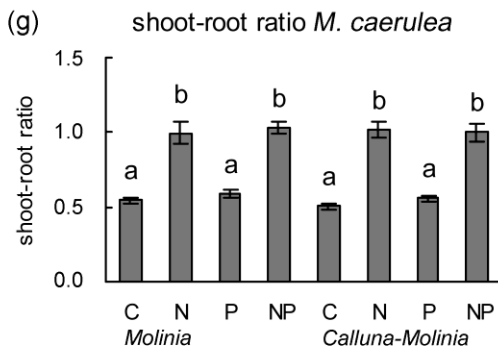
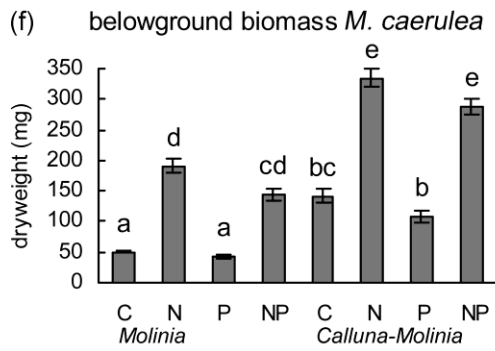
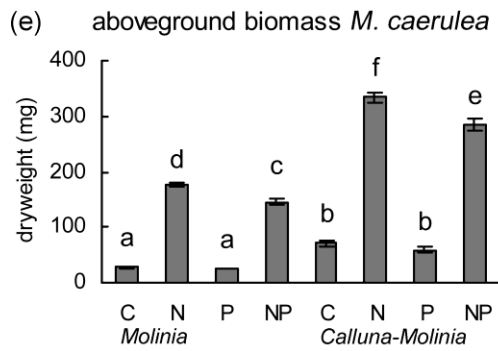
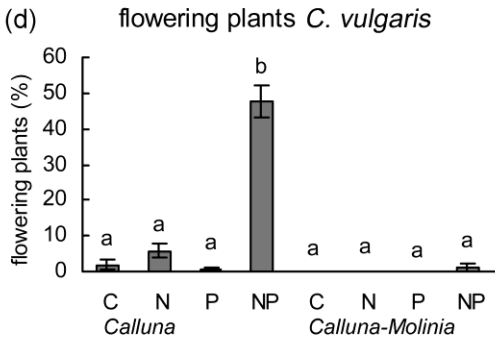
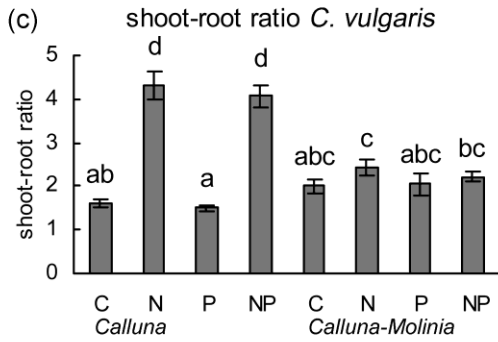
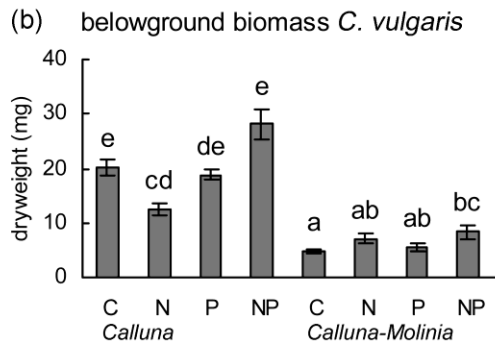
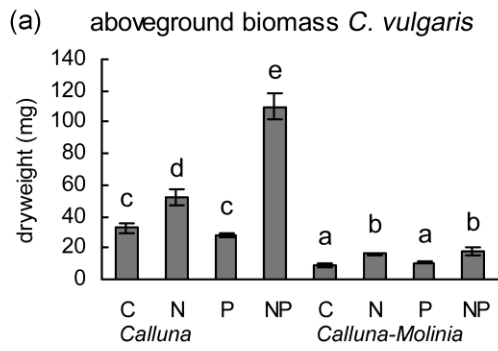


Fig. 1. Effects of fertilisation treatments (C: control; N: nitrogen; P: phosphorus; NP: nitrogen and phosphorus) and competition type (monocultures vs. mixtures) on *C. vulgaris* (*Calluna*) and *M. caerulea* seedlings (*Molinia*). Data refer to plant individuals and show mean and ± 1 standard error (error bars) of: aboveground biomass (a, e), belowground biomass (b, f), shoot-root ratios (c, g), and percentage of flowering plants (d, i) of *C. vulgaris* and *M. caerulea*, respectively. Number of tillers of *M. caerulea* is presented in Fig. 1h. Different letters indicate significant differences ($P < 0.05$).

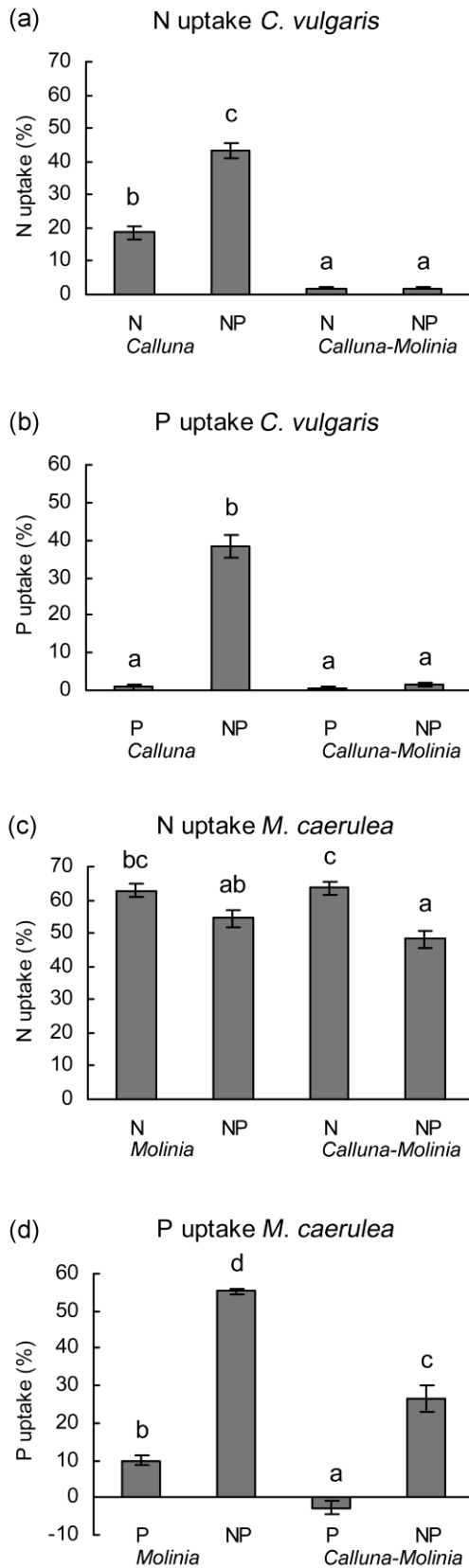


Fig. 2. Mean and ± 1 standard error (error bars) of N (a, c) and P (b, d) fertiliser uptake (%) of *C. vulgaris* (*Calluna*) and *M. caerulea* (*Molinia*), respectively. Data represent total fertiliser uptake per species and pot (i.e. of 16 plant individuals in monocultures and 8 plant

individuals in mixtures). Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA. Treatments: N: nitrogen; P: phosphorus; NP: nitrogen and phosphorus.

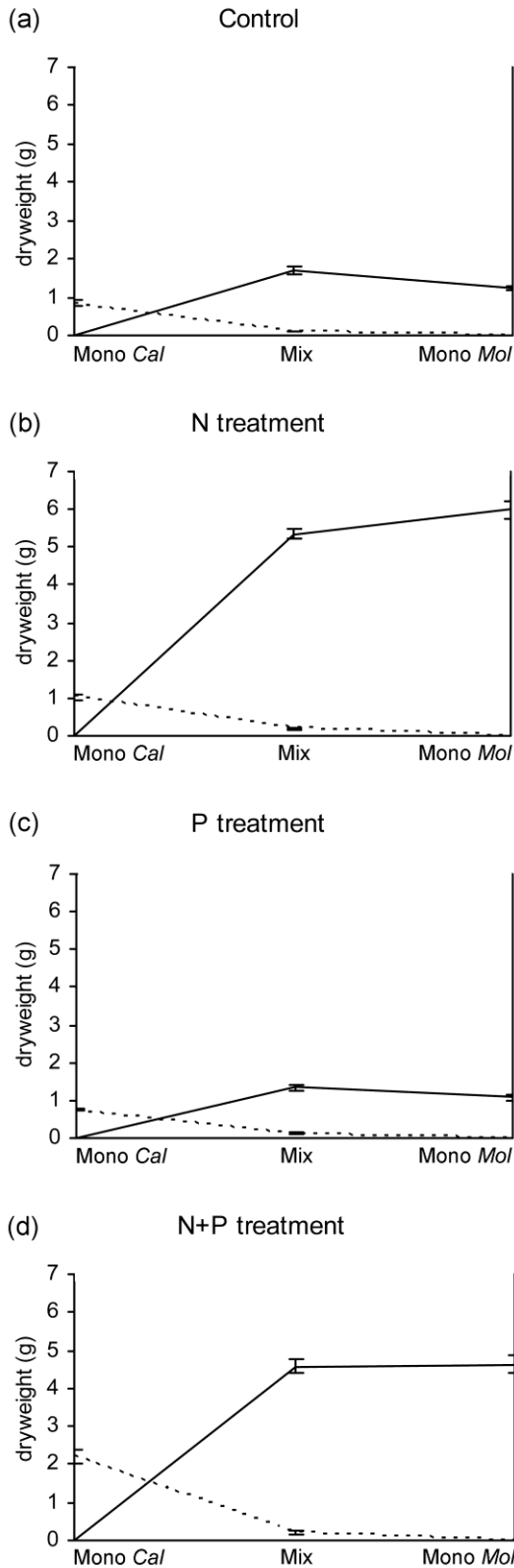


Fig. 3. Replacement diagrams based on mean biomass yields per species and pot (g dw; error bars: ± 1 standard error) for the different fertilisation treatments: control (a), nitrogen (b), phosphorus (c), nitrogen and phosphorus (d). Yields of *C. vulgaris* (dotted lines) represent the biomass of 16 plants in *C. vulgaris* monocultures (Mono Cal), 8 plants in *C. vulgaris* - *M.*

caerulea mixtures (Mix) and 0 plants in *M. caerulea* monocultures (Mono *Mo*). Yields of *M. caerulea* (solid lines) are presented in the same way.

IV

Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study

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Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study

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Abstract

In the present study, we analyze the fate of airborne nitrogen in heathland ecosystems (NW Germany) by means of a ^{15}N tracer experiment. Our objective was to quantify N sequestration and N allocation patterns in an ecosystem that is naturally limited by N, but that has been exposed to airborne N inputs exceeding critical loads for more than 3 decades. We hypothesized that the system has a tendency towards N saturation, which should be indicated by low N sequestration and high N leaching. We analyzed ^{15}N partitioning (aboveground biomass and soil horizons) and investigated ^{15}N leaching over 2 years following a ^{15}N tracer pulse addition. ^{15}N tracer recovery was 90% and 76% in the first and second year, respectively. Contrary to our expectations, more than 99% of the tracer recovered was sequestered in the biomass and soil, while leaching losses were $<0.05\%$ after 2 years. Mosses were the most important short-term sink for ^{15}N (64% recovery in the first year), followed by the organic layer. In the second year, the moss layer developed from a sink to a source (23% losses), and soil compartments were the most important sink (gains of 11.2% in the second year). Low ^{15}N recovery in the current year's shoots of *Calluna vulgaris* ($<2\%$) indicated minor availability of ^{15}N tracer sequestered in the organic layer. N partitioning patterns showed that the investigated heaths still have conservative N cycling, even after several decades of high N loads. This finding is mainly attributable to the high immobilization capacities for N of podzols in soil compartments. In the long term, the podzol A- and B-horizons in particular may immobilize considerable amounts of incoming N. Since N compounds of these horizons are not readily bio-available, podzols have a high potential to withdraw airborne N from the system's N cycle.

Keywords: *Calluna vulgaris*, dry lowland heath, N cycling, N deposition, N retention, N saturation

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Introduction

Since the beginning of the industrialization in the 19th century the deposition of reactive nitrogen compounds has tripled on a global scale (Galloway *et al.*, 2004). Airborne nitrogen loads have increased the availability of nitrogen to plants which has been observed to result in changes in species composition and losses of species diversity in many terrestrial ecosystems (Bobbink *et al.*, 1998; van Diggelen & Marrs, 2003). Heaths in particular are characterized by nutrient-poor conditions, and species typical of heaths such as *Calluna vulgaris* (henceforth referred to as *Calluna*) are well adapted to low nitrogen availability (Gimingham, 1972). Therefore, heaths are highly susceptible to airborne nitrogen inputs. Several studies found an increase in biomass production for *Calluna* and an increase in shoot nitrogen concentrations either over deposition gradients or as a

result of fertilization (Lee *et al.*, 1992; Uren *et al.*, 1997; Power *et al.*, 1998; Carroll *et al.*, 1999; Kirkham, 2001; Pilkington *et al.*, 2005). Increasing susceptibility to secondary stress factors such as frost and drought as well as increasing herbivory by insects (heather beetle) were further impacts observed (Bobbink *et al.*, 2002). The long-term effects are decreasing lichen and moss diversity, increasing cover of herbaceous species and finally a shift from dwarf shrub- to grass-dominated systems (Heil & Diemont, 1983; Carroll *et al.*, 1999; Brys *et al.*, 2005; Calvo *et al.*, 2005).

Severe shifts in the functioning of heaths were expected to occur beyond critical loads of 10–20 $\text{kg N ha}^{-1} \text{yr}^{-1}$ (Bobbink *et al.*, 2002), but underlying processes are not fully understood. For example, deposition rates in dry lowland heaths of NW Germany have exceeded critical load thresholds for 30 years (i.e. input rates $>20 \text{ kg N ha}^{-1} \text{yr}^{-1}$; Matzner, 1980; Steubing *et al.*, 1992; Niemeier *et al.*, 2005). Because of the high N loads these systems have received over 3 decades, some indications of progressing N saturation are to be

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expected. Aber *et al.* (1998) presented a hypothetical course of N saturation for forest ecosystems in which advanced stages of N saturation are characterized by reduced aboveground biomass production as well as N losses via leaching. This is in agreement with the observations reported by Verhoeven *et al.* (1996) according to which the absence of ongoing growth responses as a result of N fertilization coincides with a shift from N to P limitation. However, recent fertilization experiments have indicated that growth of *Calluna* in dry lowland heaths in NW Germany still seems to be limited by N, even after 5 years of supplemental additions of $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (von Oheimb *et al.*, 2010). Leaching losses, however, increased from $2.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the late 70s to $3.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in 10–15-year-old *Calluna* stands (Matzner, 1980; Härdtle *et al.*, 2007b). Thus, currently available data provide no clear indication as to the extent to which the heaths in our study might be N saturated. The question also remains as to the fate of the airborne nitrogen which (heathland) ecosystems in NW Europe have received over a long period and in quantities that exceeded critical load thresholds. Despite positive growth responses of *Calluna* to nitrogen additions it is hardly conceivable that the aboveground biomass serves as an important long-term sink for incoming N (Power *et al.*, 1998). N storage in the heath soil thus seems to be a plausible explanation; however, continuously increasing soil N stores should cause accelerated N cycling accompanied by improved N availability (Berendse, 1990; Tye *et al.*, 2005) and this in turn does not correspond to the N limitation which still seems to be present even after 30 years of high airborne loads.

Many recent studies have attempted to analyze and to predict heathland responses to past and present N loads by means of long-term fertilization experiments. These studies revealed an increased accumulation of litter and increasing N concentration in the organic soil layer (Power *et al.*, 1998; Carroll *et al.*, 1999; Pilkington *et al.*, 2005).

Heathlands, thus, might have a flexible storage capacity for N in different compounds and different soil horizons. Pilkington *et al.* (2005) found that the soil N pool, especially of the organic layer, of an upland heath increased with the amount of experimentally added N after 11 years of N fertilization, and only under low N treatments added N was stored in the biomass. Power *et al.* (1998) performed budget calculations for a lowland heath which received 7.7 and $15.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ during 7 years of experimental fertilizations. In the high N treatment, 18% of the N added was found in the aboveground biomass and 14% in the litter layer. Since leaching and denitrification losses were minimal, the authors concluded that the bulk of the experimentally added N has been accumulated in the remaining soil compartments (e.g. O_F , O_h - and A-horizons).

Budget calculations from fertilization experiments thus might be a helpful approach to analyze allocation patterns of airborne N in heathland ecosystems. However, the quantification of total N stores cannot explain the origin of gains and losses or retention times in a focal ecosystem compartment. ^{15}N tracer studies; however, overcome these problems, since they allow for quantifications of ecosystem N flows, as has been demonstrated in the case of forest ecosystems (Nadelhoffer & Fry, 1994; Buchmann *et al.*, 1996; Tietema *et al.*, 1998; Schleppei *et al.*, 1999; Providoli *et al.*, 2006). The amount of added ^{15}N tracer can be small enough to avoid additional disturbances of the ecosystem's N cycle, but N pathways can be traced and quantified through ecosystem compartments over time.

Quantitative analyses of the fate of airborne N loads with regard to different ecosystem compartments are important to understand ecosystem responses to long-term N inputs at the individual plant and community level. Such analyses may also allow for a better understanding of mechanisms underlying shifts in the species composition of a focal ecosystem. Moreover, information about allocation patterns of deposited N is needed to develop appropriate management strategies, which, in turn, are a prerequisite for the long-term protection of heaths and the huge amount of biodiversity they host in Europe (Maskell *et al.*, 2010). The present study aims to contribute to a better understanding of the fate of airborne N in low-N ecosystems, taking dry heathlands as an example. Our objective was to quantify N sequestration and N allocation patterns in an ecosystem that is naturally limited by N, but that has been exposed to N inputs exceeding critical load thresholds for more than 3 decades. To this end, a ^{15}N tracer experiment was carried out (by means of a tracer pulse addition in early summer) in dry lowland heaths in NW Germany. We analyzed ^{15}N allocation patterns in the aboveground biomass (current year's shoots, 1–2-year-old shoots) and in soil compartments (organic layer, A- and B-horizons) as well as ^{15}N leaching losses during two growing seasons. We hypothesized that the system has a tendency towards N saturation which is expressed in the form of low N sequestration and high N leaching rates. These expectations are based on the nitrogen saturation hypothesis of Aber *et al.* (1998) and Bobbink's Critical Loads Concept (Bobbink *et al.*, 2002). Our questions were: (i) What is the fate of airborne N within 2 years after ^{15}N tracer addition and which ecosystem compartments (biomass, soil) are the most important sinks for sequestered N? (ii) Are there indications of a beginning N saturation? (iii) Are there any indications of long-term sinks in the soil that may diminish the quantity of N in the system's N cycle?

Materials and methods

Study site

Our study area is located in Lower Saxony (NW Germany) and belongs to the Lüneburger Heide nature reserve, which comprises a heathland area 5000 ha in size. This area is characterized by Pleistocene sandy deposits, and the prevailing soil types are nutrient-poor podzols. The climate is of a humid suboceanic type with a mean precipitation of 811 mm yr⁻¹ and a mean temperature of 8.4 °C (Niemeyer *et al.*, 2005). The background deposition in the study area was determined to be in the range of 20.5–25.0 kg N ha⁻¹ yr⁻¹ (Härdtle *et al.*, 2007b).

Study design

In the study area, a series of seven replicated plots was selected at random. *Calluna* was monodominant (cover > 80%) in all plots. The age of *Calluna* ranged between 10 and 12 years and all plots had a well developed moss layer (mean cover 80–100%) dominated by *Hypnum cupressiforme* or *Pleurozium schreberi*. Plots comprised two subplots, each of which was 8 m² in size (2 m × 4 m). One subplot received ¹⁵N tracer (henceforth referred to as 'labeled subplot') and the other was used for the determination of ¹⁵N natural abundance (henceforth referred to as 'nonlabeled subplot'). Labeled and nonlabeled subplots were separated by a buffer zone 1 m in width to avoid cross contamination after ¹⁵N tracer addition.

For the calculation of leaching losses, two lysimeters per plot were installed at a distance of 2 m from the plots. One lysimeter received ¹⁵N tracer (henceforth referred to as 'labeled lysimeter') and the other was used for the determination of ¹⁵N natural abundance (henceforth referred to as 'nonlabeled lysimeter'). Only five of seven plots were equipped with lysimeters, as sampling and checking these devices is a time-consuming process. PVC pipes (90 cm length and 50 cm in diameter) were slowly hammered into the soil. The surrounding soil was removed consecutively, so that the pipe finally contained an undisturbed soil core covered by *Calluna* (DVWK, 1980). The bottom end of the pipe was then sealed and made air-tight with a PVC lid (with outlets for the seepage water connected to a pump), and then buried at the same location. A porous disc (PE-sinter; ecoTech, Bonn, Germany) covered by a nylon membrane (pore diameter 0.45 µm; Whatman Ltd., Maidstone, UK) was installed at the bottom of each lysimeter. All the seepage water leached through the lysimeter was sampled by means of a tension-controlled pump (-200 mbar) and collected continuously in glass bottles. Plots and lysimeters were fenced in to prevent grazing.

¹⁵N tracer addition

In the second week of June 2007, pulse labeling (Turner & Henry, 2009) with ¹⁵NH₄¹⁵NO₃ (98 at.%) was performed in all labeled subplots and labeled lysimeters. ¹⁵N addition was carried out with a special spray bottle equipped with a nozzle that allowed for an evenly distributed addition of the ¹⁵N tracer to the subplots and lysimeter surfaces. During this

procedure, *Calluna* twigs were lifted to avoid foliar contact and thus direct uptake of ¹⁵N by leaves. Labeled subplots and lysimeters received 100 mg ¹⁵NH₄¹⁵NO₃ m⁻² dissolved in 0.5 L deionized water. This quantity aimed at a target δ¹⁵N of 500‰ in *Calluna* and corresponded to 1.6% of the current annual airborne N loads. Therefore, no fertilization effect was expected. After tracer addition, the same amounts of deionized water were sprayed a second time to rinse vegetation surfaces and to disperse tracer solution. Nonlabeled subplots and nonlabeled lysimeters received (area-related) the same amounts of water without ¹⁵N tracer.

Biomass and soil sampling

From June 2007 to November 2008, we sampled the following compartments of the ecosystem: mosses, the current year's and 1–2-year-old shoots of *Calluna*, the organic layer, the albic, and the spodic horizon (i.e. O-, A- and B-horizon of podzols; FAO, 2006). Samples were collected on 11 occasions during the growing season (i.e. 2, 3, 4, 5, 10, 14, 19, 40, 51, 62, 73 weeks after ¹⁵N tracer addition; for exact sampling dates; see Table 1). Samples were taken from labeled and nonlabeled subplots on each occasion.

Twenty randomly chosen current year's shoots from the top of randomly chosen *Calluna* plants were cut with scissors from the whole subplot area and bulked to one sample. In March 2008, no current year's *Calluna* shoots were sampled, because *Calluna* does not start annual shoot growth until the end of May at the earliest. In addition, 1–2-year-old shoots were collected in the same way on each occasion. Moss samples (squares, 2 cm × 2 cm in size) consisted of six randomly chosen samples of the moss layer per subplot. Soil samples were collected from six randomly chosen locations per subplot. Squares of 2 cm × 2 cm were cut with a knife and comprised the entire depth of the organic horizon. Fresh fallen litter was excluded. A- and B-horizons were sampled from soil cores drilled with a soil auger (Pürckhauer, eco Tech).

Leachate sampling

Leachate from lysimeters was collected continuously over the growing season. Samples from labeled and nonlabeled lysimeters were taken at intervals depending on rain events (*n* of sampling occasions was 21). Total amounts of leachate were recorded for each sampling location. Simultaneously, precipitation data (mean monthly precipitation) were obtained from the German Weather Service (DWD, Hamburg, Germany) in order to assess precipitation effects on leaching patterns. During the winter months no sampling took place, because leachate was frozen in collecting flasks.

N contents and ¹⁵N analysis in biomass, soil and leachate

Plant samples were air-dried and sheared with an ultracentrifugal mill (ZM 200, Retsch, Haan, Germany). Soil samples were stored in a freezer (-18 °C) until analysis. Before analysis, samples were air-dried, sieved (2 mm) and ground with a mixer mill (MM 400, Retsch). Milled plant and soil samples

1552 U. FRIEDRICH *et al.***Table 1.** ^{15}N abundances in nonlabeled (Ref) and labeled subplots (^{15}N) of the heath compartments analyzed after ^{15}N tracer addition

Date of sampling	Time (weeks after ^{15}N addition)	Moss		Current year's shoots		1–2 year shoots		O-horizon		A-horizon		B-horizon	
		Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N	Ref	^{15}N
2007													
06/25	2	–5.7 (0.1)	788.3 (140.5)	–5.6 (0.5)	105.4 (30.0)	–6.5 (0.4)	91.5 (34.5)	–4.4 (0.3)	18.8 (6.3)	5.8 (0.2)	9.8 (0.5)	8.6 (0.5)	9.9 (0.2)
07/02	3	–5.7 (0.1)	836.1 (120.7)	–5.6 (0.4)	75.4 (13.6)	–6.3 (0.6)	60.3 (9.5)	–3.8 (0.4)	12.4 (5.1)	5.5 (0.3)	8.4 (0.6)	8.3 (0.5)	8.9 (0.5)
07/09	4	–5.9 (0.3)	918.2 (136.0)	–5.4 (0.5)	129.9 (30.2)	–6.3 (0.4)	103.7 (27.5)	–4.0 (0.3)	30.7 (7.8)	5.2 (0.2)	10.0 (1.1)	8.6 (0.5)	9.7 (0.6)
07/16	5	–5.8 (0.2)	852.5 (104.3)	–5.1 (0.4)	166.6 (33.5)	–6.6 (0.3)	117.4 (27.9)	–4.2 (0.4)	11.4 (4.6)	6.5 (0.2)	11.3 (1.9)	8.7 (0.4)	10.4 (0.4)
08/22	10	–6.1 (0.2)	719.9 (91.1)	–5.4 (0.5)	122.7 (22.6)	–5.7 (0.5)	110.9 (19.7)	–4.5 (0.3)	17.7 (4.1)	5.3 (0.3)	7.8 (0.6)	8.5 (0.4)	8.4 (0.7)
09/22	14	–6.0 (0.2)	602.1 (44.3)	–5.7 (0.4)	160.9 (27.2)	–5.6 (0.6)	128.6 (22.1)	–4.4 (0.4)	15.7 (4.6)	4.7 (0.6)	10.7 (1.8)	8.3 (0.5)	8.8 (0.5)
10/26	19	–6.1 (0.2)	877.5 (123.2)	–5.1 (0.5)	122.9 (25.6)	–5.8 (0.5)	85.9 (12.9)	–4.2 (0.3)	25.0 (5.6)	5.4 (0.6)	8.1 (1.1)	8.3 (0.4)	8.7 (0.6)
2008													
03/21	40	–5.2 (0.2)	577.8 (54.8)	ND ND	ND ND	–6.5 (0.3)	62.8 (15.8)	–4.1 (0.3)	26.2 (8.0)	5.5 (0.3)	8.2 (0.8)	8.6 (0.3)	9.0 (0.8)
06/05	51	–5.5 (0.1)	615.4 (85.3)	–5.8 (0.5)	60.1 (12.6)	–6.8 (0.4)	59.5 (17.0)	–4.0 (0.4)	27.7 (7.3)	4.9 (0.2)	10.7 (1.2)	8.4 (0.3)	9.7 (0.9)
08/25	62	–5.5 (0.2)	529.3 (53.6)	–5.6 (0.5)	58.9 (12.2)	–6.8 (0.4)	50.7 (12.8)	–4.4 (0.4)	28.0 (5.9)	5.8 (0.2)	9.2 (0.9)	8.2 (0.6)	9.2 (0.6)
11/04	73	–5.2 (0.2)	457.4 (44.0)	–5.9 (0.5)	66.6 (9.7)	–6.6 (0.5)	55.4 (10.1)	–4.1 (0.4)	26.0 (5.4)	4.9 (0.6)	8.3 (0.9)	8.4 (0.4)	9.1 (0.5)

Data are means of $\delta^{15}\text{N}$ (‰) with 1 SE in parentheses ($n = 7$; time in weeks after ^{15}N tracer addition; current year's shoots in March 2008 not determined (ND) due to the annual start of shoot growth in April/May; negative values indicate a depletion, positive values an enrichment of ^{15}N in the $^{15}\text{N}/^{14}\text{N}$ ratio in the sample compared with atmospheric N_2).

were stored at room temperature and redried at 105 °C before weighing. Total N and $\delta^{15}\text{N}$ were determined using a continuous flow elemental analyzer-isotopic ratio mass spectrometer (vario El cube, Elementar, Hanau, Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK).

Leachate was filtered, stored in a freezer (–18 °C) and defrosted at a temperature below 4 °C before analysis. NO_3^- -N contents of leachate samples were analyzed using an ion exchange chromatograph (DX-120, Dionex, Idstein, Germany; prestudies revealed negligible low NH_4^+ -N leaching). For determination of $\delta^{15}\text{N}$: leachate samples were prepared following a modified diffusion method (Sebilo *et al.*, 2004), and subsequently analyzed as described above.

Calculation of N pools and N leaching losses

Two of the 11 sampling occasions (August 2007 and 2008) were chosen as reference dates in order to compare ^{15}N tracer recovery for both years. A prerequisite for the calculation of the ^{15}N tracer recovery is the knowledge of the N pool sizes (total amount of N in a particular compartment) of labeled

subplots for all the compartments analyzed. N pools were calculated by means of the pool masses multiplied by their N contents. If compartment N contents between the two reference dates differed significantly (paired *t*-test, $P < 0.05$), N pool sizes were calculated separately for each date, based on the corresponding result for the N content. If differences were nonsignificant, means of N contents were used, resulting in the same N pool size for both reference dates (cf. Table 2).

Total pool mass of the moss compartment was calculated using the mean dry weight of mosses (per unit area) and the individual moss cover from each labeled subplot. In September 2007, *Calluna* aboveground biomass was harvested at sites 0.25 m² in size and situated near the plots. Biomass was separated into current year's shoots, 1–2-year-old shoots and remaining older biomass. For the current year's shoots as well as for 1–2-year-old shoots dry weights were determined after drying at 80 °C. Means of dry weights were used as the total pool mass of current year's shoots and 1–2-year-old shoots. Total soil masses of the O-, A- and B-horizon were calculated by means from the thickness of a soil horizon and its bulk density. Bulk densities of soil horizons of the podzols typical of the study area were taken from former analyses (Niemeyer

Table 2 Soil depth, mass, N content and ¹⁵N tracer recovery of the heath compartments analyzed for two late-summer dates (August 2007 and 2008)

Compartment	Soil depth (cm)	2007				2008				
		Mass (kg m ⁻²)	N content (%)	N pool (g N m ⁻²)	¹⁵ N _{rec} (mg N m ⁻²)	% ¹⁵ N _{rec}	N content (%)	N pool (g N m ⁻²)	¹⁵ N _{rec} (mg N m ⁻²)	% ¹⁵ N _{rec}
Moss		0.53 (0.01)	1.60 (0.09)	8.40 (0.50)	22.71 (3.18)	64.21 (8.99)	1.36* (0.06)	7.17 (0.35)	14.48 (2.00)	40.93* (5.65)
Current year's shoots		0.08 (0.01)	1.48 (0.07)	1.15 (0.05)	0.57 (0.13)	1.62 (0.35)	1.24* (0.03)	0.23 (0.02)	0.23 (0.04)	0.65* (0.12)
1-2 yr shoots		0.07 (0.00)	1.01 (0.05)	0.71 (0.03)	0.32 (0.06)	0.90 (0.18)	0.82* (0.03)	0.57 (0.02)	0.12 (0.03)	0.35* (0.08)
O-horizon	2.73 (0.20)	4.37 (0.44)	1.88 (0.07)	83.32 (10.39)	6.46 (1.10)	18.26 (3.10)	1.88 (0.08)	83.32 (10.39)	9.25 (1.55)	26.16 (4.39)
A-horizon	7.06 (0.97)	165.05 (27.13)	0.11 (0.02)	189.72 (42.13)	1.47 (0.44)	4.16 (1.25)	0.11 (0.01)	189.72 (42.13)	2.48 (0.44)	7.02* (1.25)
B-horizon	7.64 (0.94)	89.42 (14.91)	0.10 (0.01)	98.78 (33.11)	0.15 (0.08)	0.41 (0.23)	0.10 (0.03)	98.78 (33.11)	0.30 (0.09)	0.84* (0.26)
Leaching losses					0.05	0.03			0.09	0.05
Total recovery (%)						89.58				76.00

Data are means with 1 SE in parentheses. ¹⁵N tracer recovery is expressed as total mass of ¹⁵N tracer recovered (¹⁵N_{rec}) and as percent of total ¹⁵N tracer masses (% ¹⁵N_{rec}). Leaching losses are given as sum of ¹⁵N leaching losses since ¹⁵N tracer addition.

*Significant differences of ¹⁵N tracer recovery between August 2007 and 2008 (*P* < 0.05, paired *t*-test).

et al., 2005). The thickness of soil horizons was recorded using a soil corer. Averages from three measurements per plot were used.

N leaching losses were calculated from total amounts of leachate multiplied by NO₃-N contents from labeled lysimeters.

Calculation of ¹⁵N abundance, ¹⁵N enrichment and ¹⁵N tracer recovery

¹⁵N contents from labeled and nonlabeled subplots are referred to as ¹⁵N abundances and ¹⁵N natural abundances, respectively, and are presented in the δ notation:

$$\delta^{15}\text{N}(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1]1000, \quad (1)$$

where *R*_{sample} and *R*_{standard} are the ratios between ¹⁵N and ¹⁴N of the sample and the standard, respectively. By convention, atmospheric N₂ is used as standard (δ¹⁵N = 0 according to 0.3663 at.%; Coplen et al., 1992).

¹⁵N enrichment expresses the per mille isotope enrichment in a sample from a labeled subplot (δ¹⁵N_{sample}) vs. a reference sample from a nonlabeled subplot (δ¹⁵N_{ref}) (Fry, 2006):

$$^{15}\text{N enrichment (‰)} = \frac{\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{ref}}}{\delta^{15}\text{N}_{\text{ref}} + 1000} 1000. \quad (2)$$

¹⁵N tracer recovery in compartment N pools and ¹⁵N leaching losses were calculated as follows:

$$^{15}\text{N}_{\text{rec}} = m_{\text{pool}} \frac{\text{at.}\%^{15}\text{N}_{\text{pool}} - \text{at.}\%^{15}\text{N}_{\text{ref}}}{\text{at.}\%^{15}\text{N}_{\text{tracer}} - \text{at.}\%^{15}\text{N}_{\text{ref}}}, \quad (3)$$

where ¹⁵N_{rec} is the mass of ¹⁵N tracer recovered in the N pool of labeled subplots or in leachate losses from labeled lysimeters (g N m⁻²), *m*_{pool} is the mass of the N pool of labeled subplots or the amount of total N leaching losses from labeled lysimeters (g N m⁻²), at.%¹⁵N_{pool} is the at.%¹⁵N in the N pool of labeled subplots or in leachate losses from labeled lysimeters, at.%¹⁵N_{ref} is the at.%¹⁵N in the N pool of nonlabeled subplots or in leachate from nonlabeled lysimeters, and at.%¹⁵N_{tracer} is the at.%¹⁵N of the added ¹⁵N tracer (Nadelhoffer et al., 2004). Means of at.%¹⁵N_{ref} from each compartment analyzed were tested for differences between the two reference dates in August 2007 and 2008 using paired *t*-tests (*P* < 0.05). If differences were nonsignificant, means of at.%¹⁵N_{ref} were used for the calculation of the compartment ¹⁵N tracer recovery. ¹⁵N tracer recoveries in percent (%¹⁵N_{rec}) represent masses of ¹⁵N tracer recovered as percent of total ¹⁵N tracer masses added to the labeled subplots or to labeled lysimeters.

Statistical analyses

Differences between ¹⁵N abundances of labeled and ¹⁵N natural abundances from nonlabeled subplots were tested by means of δ¹⁵N for each sampling occasion using independent *t*-tests (*P* < 0.05). Outliers defined by boxplots were omitted (<3% of the total data set). ¹⁵N leaching losses were determined by ¹⁵N tracer recovery in leachate from labeled lysimeters and presented as a cumulative curve for the 2-year period. Differences between ¹⁵N tracer recovery of the two reference dates in August 2007 and 2008 were tested using paired *t*-tests (*P* < 0.05). Shifts of ¹⁵N tracer recovery patterns

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were calculated as differences between ^{15}N tracer recovery of the two reference dates. All statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

^{15}N abundances in biomass and soil

Means of $\delta^{15}\text{N}$ in the nonlabeled subplots ranged from -6.8‰ in 1–2-year-old *Calluna* shoots to 8.7‰ in the B-horizon (Table 1). ^{15}N natural abundances of soil compartments increased with depth from the O- to the B-horizon, whereas ^{15}N natural abundances in the biomass were ^{15}N depleted in comparison to the soil.

$\delta^{15}\text{N}$ values increased in all compartments of the labeled subplots (with the exception of the B-horizon) only 2 weeks after ^{15}N addition (end of June). The increase was highest in the moss layer with a peak 4 weeks after ^{15}N tracer addition (i.e. beginning of July 2007; $\delta^{15}\text{N} = 918.2\text{‰}$). Differences between corresponding compartments of non-labeled and labeled subplots were significant ($P < 0.05$) for biomass compartments and the O-horizon on all sampling occasions. For the A-horizon, differences were significant with the exception of one sampling occasion (October 2007). For the B-horizon, differences were significant for two of the 11 sampling occasions (June 25 and July 16; results not shown in Table 1).

^{15}N enrichment in biomass and soil

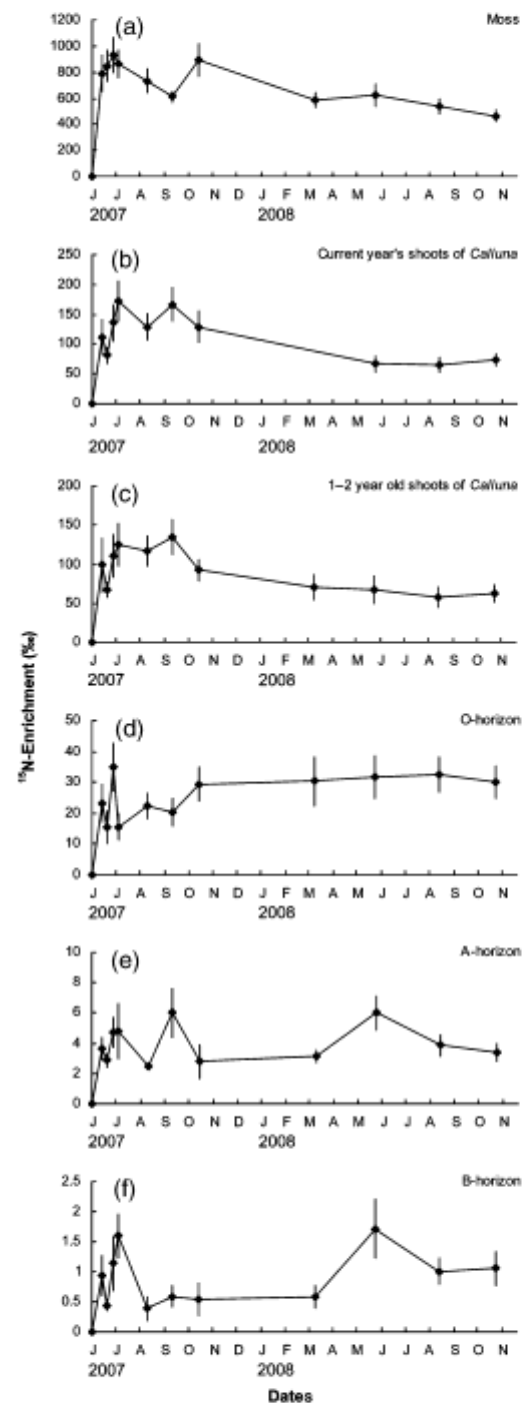
^{15}N enrichments showed a typical enrichment pattern for most of the compartments in the course of the experiment (Fig. 1). Mosses achieved the highest values, ranging between 465.0‰ and 929.5‰ . ^{15}N enrichment in the current year's shoots of *Calluna* was slightly higher than in 1–2-year-old shoots, altogether ranging between 57.9‰ and 172.3‰ . While the O-horizon achieved ^{15}N enrichments from 15.4‰ to 34.8‰ values were lower for the A- and B-horizon (0.4‰ – 6.0‰).

^{15}N enrichments showed a first maximum in all compartments 4–5 weeks after tracer addition (until July 16, 2007). After 3–4 months, ^{15}N enrichments continuously declined in the aboveground biomass (i.e. in the moss layer and the *Calluna* shoots), whilst values of all soil horizons showed a slight, but continuous tendency to increase in 2008.

Fig. 1 ^{15}N enrichment (‰) of the heath compartments analyzed for 11 sampling occasions following ^{15}N tracer addition. (a, moss layer; b, current year's shoots; c, 1–2-year-old shoots; d, O-horizon; e, A-horizon; f, B-horizon). It has to be noticed, that N pool sizes are not taken into account when calculating ^{15}N enrichments. Therefore, ^{15}N enrichments are not reflecting absolute ^{15}N tracer retentions or absolute fluxes between different compartment N pools.

^{15}N leaching losses

Total ^{15}N losses via leaching were negligible compared with the sequestration of ^{15}N in the aboveground



biomass and soil (about 0.05%; expressed as a cumulative curve in Fig. 2). Slight losses appeared within a period of 4–5 weeks (until the middle of July) immediately after tracer addition (with a perceivable lag phase during the first 2 weeks), but then remained very low for the remainder of the experiment. Notably, highest leaching losses during the first 4–5 weeks corresponded with maximum values for ¹⁵N enrichments in all compartments. Since the courses of monthly precipitation rates were well comparable for both years, there was no annual effect of precipitation on leaching rates.

¹⁵N tracer recovery

Recovery of ¹⁵N was highest for the moss layer, but there was a distinct decrease in ¹⁵N recovery in this compartment from 64% in 2007 to 41% in 2008 (Table 2,

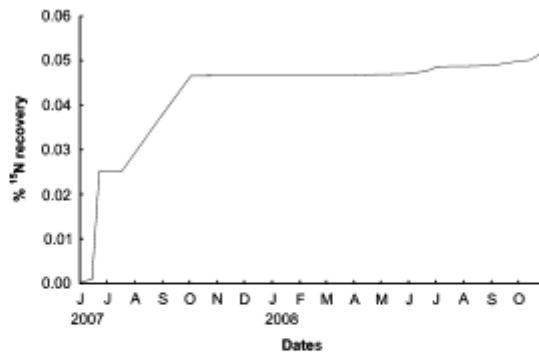


Fig. 2 ¹⁵N leaching losses expressed as a cumulative curve of ¹⁵N recovery (%).

Fig. 3). Recovery in current year’s shoots was higher than in 1–2-year-old shoots and ranged between 0.35% and 1.62% for both years. Decreases in the current year’s and 1–2-year-old shoots of *Calluna* were 1.0% and 0.6% from 2007 to 2008, respectively. A decrease in ¹⁵N recovery in biomass compartments (about 25%, Fig. 3) corresponded with an increase in soil compartments, but total losses from the aboveground biomass were higher than gains in soil compartments (leaching losses included; Fig. 3). ¹⁵N recovery in the O-horizon was 23.3% in 2007 and increased by 7.9% in 2008 (Fig. 3). In tendency, recovery in soil compartments decreased with depth, but recovery rates for all soil compartments significantly increased in 2008 (recovery in the A- and B-horizons approximately doubled in 2008). Total ¹⁵N tracer recovery for all compartments (including leaching losses) was 90% in 2007 and 76% in 2008.

Discussion

¹⁵N partitioning and recovery

Contrary to our expectations, the heaths studied still showed high sequestration and retention capacities for incoming N. This was indicated by both a high recovery in the biomass and soil compartments and negligible leaching losses. Thus, the focal heaths still exhibit characteristics of conservative N cycling (Tye *et al.*, 2005).

The bryophyte layer proved to be the major short-term sink for N, but became a source in 2008 (Fig. 3). This result indicates that mosses function as an important compartment responsible for the sequestration of

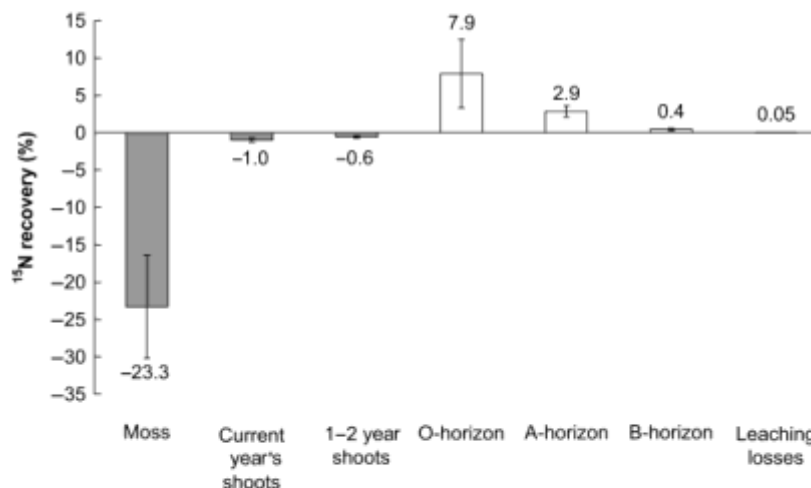


Fig. 3 Shifts in ¹⁵N tracer recovery patterns of heath compartments from August 2007 to 2008. Data represent differences in ¹⁵N tracer recovery (%) between the two dates. Leaching losses are the sum of total ¹⁵N tracer losses since ¹⁵N tracer addition in percent of added ¹⁵N tracer.

airborne N into the heath's N cycle, because incoming N is rapidly immobilized, but may be successively released in the following weeks or months. Since bryophytes are ectohydric plants (i.e. the cell surface absorbs dissolved nutrients; Proctor, 2008), enrichments for the moss layer comprised both nitrogen adhered to the moss plant's surface and assimilated nitrogen. In addition, it is conceivable that the microbial communities associated with the moss phytomass were also responsible for the immobilization of N (Tye *et al.*, 2005). These processes contribute to an immediate sequestration of N. The importance of cryptogams (including lichens) in the sequestration and partitioning of airborne N loads has also been shown for other ecosystems such as arctic tundra or arctic heaths (Gordon *et al.*, 2001; Tye *et al.*, 2005). Since airborne N inputs are the main N source for bryophytes (Bates, 2008), and airborne N has to pass the moss compartment before entering the soil, we suppose that ^{15}N tracer retention found for the moss compartment mirrors the natural partitioning processes. In this context it is important to note that high uptake capacities of the moss compartment are indicative of a low N saturation level (Curtis *et al.*, 2005).

In addition to the moss layer, tracer recovery was high for the O-horizon, but with distinctly increasing values in the second year. It is likely that the rapid sequestration of applied inorganic N in the O-horizon is mainly attributable to the microbial biomass present in this horizon. Jonasson *et al.* (1996) and Nordin *et al.* (2004) highlighted the importance of soil microbes with regard to a rapid and substantial immobilization of both inorganic and organic N compounds in soils. It is also presumed that proteins from microbial biomass act as precursors of recalcitrant soil organic matter and constitute the stable organic N pool of soils (Högberg, 1997; Hagedorn *et al.*, 2005; Horwath, 2007). High N retention in the O-horizon in heaths was also reported in other studies (Kristensen & McCarty, 1999; Kristensen, 2001; Pilkington *et al.*, 2005), but has never been quantified by means of ^{15}N tracer field experiments. In forest ecosystems, however, where several ^{15}N tracer studies took place, comparable N allocation patterns were found. In all studies the organic soil was the most important sink for added ^{15}N (Buchmann *et al.*, 1996; Tietema *et al.*, 1998; Nadelhoffer *et al.*, 2004; Providoli *et al.*, 2006).

Regarding the amounts of ^{15}N tracer recovered in the O-horizon, high ^{15}N tracer uptake by *Calluna* was expected, since its roots are mainly located in the O-horizon (Gimingham, 1972), and *Calluna* can utilize both inorganic and organic N compounds (due to its ericoid mycorrhiza; Read, 1991). However, comparison of ^{15}N recovery in *Calluna* shoots and in the O-horizon does not confirm this assumption. The current year's shoots of *Calluna* incorporated 0.23 mg N m^{-2} of ^{15}N until August 2008,

while 9.25 mg N m^{-2} remained in the O-horizon (Table 2). Thus, only a minor proportion of ^{15}N tracer from the O-horizon was available to plants. There may be two reasons for this: ^{15}N recovered in the O-horizon mainly belonged to a stable N pool not available to *Calluna* [i.e. slow cycling fractions of dissolved organic nitrogen (DON); Compton & Boone, 2002; Currie *et al.*, 2004; Jones *et al.*, 2004], or *Calluna* was the weaker competitor for N in comparison to the soil microbial communities (Schimel & Chapin, 1996; Schimel & Bennett, 2004). This may also indicate that a large proportion of the O-horizon's ^{15}N was immobilized by the microbial biomass (Kristensen & McCarty, 1999).

In our experiment, it is likely that increasing ^{15}N recovery in the O-horizon in 2008 was attributable to influxes of the moss compartment, but the chemical form of translocated N remains unclear. Since decomposition processes of the moss biomass are too slow, N losses from the moss compartment cannot be explained by this process alone. Other processes such as leaking cells (as a result of desiccation–rehydration events; Bates, 2008) could also contribute to ^{15}N tracer losses.

In addition to the O-horizon, ^{15}N recovery significantly increased in the A- and B-horizon during the second year. These shifts in recovery rates may indicate that an imbalance of ^{15}N fluxes from the aboveground biomass to the soil still exists, in which the importance of the A- and B-horizons as long-term sinks for N may increase with time. Considering both the doubling of ^{15}N recoveries in the second year and the huge N stores in the A- and B-horizons (about 190 and 99 g N m^{-2} , respectively; Table 2), it is likely that the podzol A- and B-horizons in particular can accumulate considerable amounts of N (Nielsen *et al.*, 2000). In addition, the formation and downward translocation of fulvic and humic acids (as organometallic compounds including considerable amounts of N) is a typical process that takes place in podzols (Brady & Weil, 2001; FAO, 2006). Since these compounds are insoluble to a great extent and not readily bio-available (Hagedorn *et al.*, 2005), N located in the A- and B-horizons may be partly withdrawn from the system's N cycle. Podzols thus have the potential to immobilize airborne N due to the long-term sequestration in their A- and B-horizons. This process may contribute to the phenomenon that dry heaths still appear to be N limited, and thus to immobilize high amounts of airborne N, even after decades of atmospheric inputs above critical loads. Thus, the soil horizons typical of podzols (i.e. the organic layer and the albic horizon, both forming the so-called raw humus, and spodic horizons) may serve as the most important long-term sinks for N in dry heaths.

Our findings in principle support Aber's revised nitrogen saturation hypothesis (Aber *et al.*, 1998), according to which high N leaching proves to be a final

response mechanism of an ecosystem to high and chronic inputs of N (so-called 'stage 3' of Aber's revised hypothesis; in contrast to his initial model, in which significant N leaching already appeared at the second stage of 'integrated responses of nitrogen-limited forests to chronic nitrogen additions'; Aber *et al.*, 1989). Thus, ecosystem responses to N inputs occur at different time scales (Bobbink *et al.*, 2002). Our findings, however, indicate that response patterns are strongly mediated by ecosystem properties such as soil types and characteristic soil horizons (e.g. thickness of the humus-horizons) as well as the species composition (e.g. the presence of a moss cover, successional stage of a heath). This may lead to an underestimation of the ecosystem's resilience towards long-term inputs of N, because the extent to which airborne N may be immobilized in an ecosystem compartment (e.g. podzol humus-horizons) is difficult to predict. This may also hamper the practical applicability of the Critical Load Concept, because the susceptibility of heathlands to chronic N inputs may vary in a wide range corresponding to the variability of soil conditions (which is currently mirrored by the wide range of critical loads between 10 and 20 kg N ha⁻¹ yr⁻¹ given for dry heaths; Bobbink *et al.*, 2002).

¹⁵N leaching losses and not quantified losses

Contrary to our expectations, ¹⁵N leaching losses were negligible in the course of the experiment. After a lag phase of 2 weeks (corresponding with the downward transport of applied tracer to the lower end of the lysimeter), highest leaching losses appeared within the first 5 weeks of the experiment (until the middle of July). These losses were due to the immediate leaching of small amounts of ¹⁵N tracer that had not been immobilized in the moss layer, by soil microbes or due to plant uptake. In our experiment, low leaching losses of N corresponded with high immobilization rates found for the moss layer and the O-horizon. In heaths, leaching losses are low as long as sites are undisturbed, but may increase as a result of disturbance such as heather beetle infestations or high-intensity management measures (Nielsen *et al.*, 1999, 2000; Härdtle *et al.*, 2007b). In the Netherlands, however, high leaching losses were found as a result of high N deposition rates and interpreted as an indication of N saturation (Schmidt *et al.*, 2004).

We assume that the highest proportion of not recovered ¹⁵N was accumulated in compartments that were not sampled in this study (i.e. aboveground biomass of *Calluna* older than 2 years or roots). In addition, N losses attributable to denitrification, volatilization and DON leaching were not quantified. However, we expect

denitrification losses to be of minor importance in dry heaths (Power *et al.*, 1998; Kristensen & McCarty, 1999; Härdtle *et al.*, 2007a), and losses by volatilization were also expected to be low in strongly acid soils. We also assume leaching of DON to be small (Nielsen *et al.*, 1999, 2000). According to Aber *et al.* (1998), N losses due to leaching of DON are in the range between 0.3 and 0.5 g m⁻² yr⁻¹ (in forest ecosystems under low and high N input rates). Owing to ground frost events lysimeters were not operated in winter, but winter losses should be low, since the downward movement of seepage water during frost events is limited and winter concentrations of dissolved inorganic N are lower than values found for the growing season (Härdtle *et al.*, 2007b).

Conclusions

The present study indicates that the dry lowland heath investigated is still limited by N, despite 30 years of ongoing high nitrogen deposition. This was indicated by high immobilization rates and negligible ¹⁵N leaching losses (about <0.05% of the total ¹⁵N tracer recovery after 2 years). The moss compartment served as the major short-term sink, but became a source in the second year. Bryophytes are, thus, an important ecosystem component responsible for the sequestration of airborne N into the heath's N cycle (rapid immobilization of incoming N, but successive N release in the following weeks or months). In the course of the experiment ¹⁵N recovery decreased in the aboveground biomass, but increased in all soil compartments. This indicates that an imbalance of ¹⁵N fluxes from the aboveground biomass to the soil continued to exist throughout the experiment. It is likely that the formation and downward translocation of ¹⁵N (e.g. as organometallic compounds) will continue in the future. Ecosystems associated with podzols may thus have the potential to immobilize airborne N loads due to their long-term sequestration in the podzol A- and B-horizons. This process may also explain why the heaths studied still exhibit conservative N cycling (high sequestration rates, no leaching losses), even after a long-term history of airborne N loads above critical loads.

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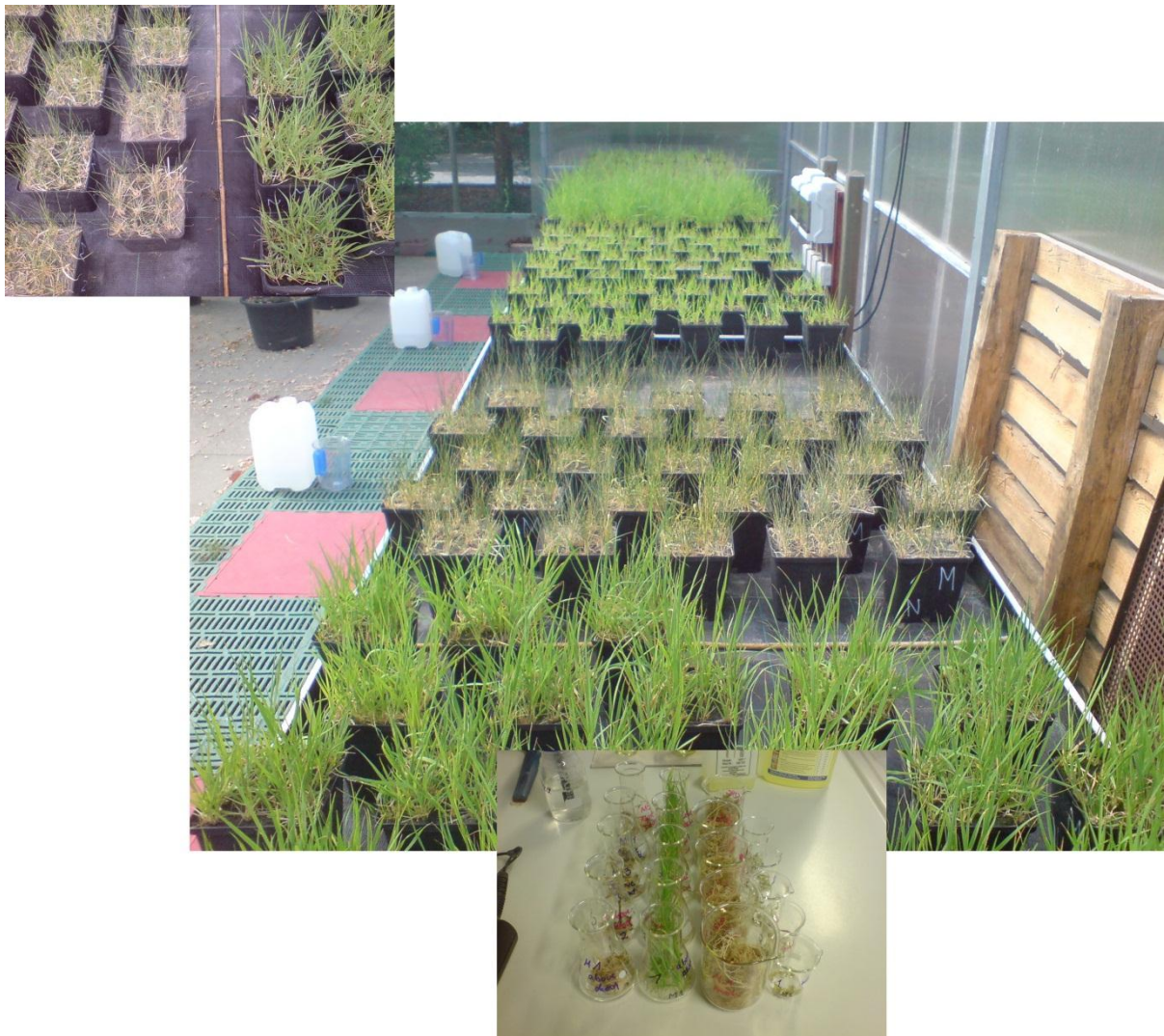
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Nitrogen deposition increases susceptibility to drought - experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench

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**Nitrogen deposition increases susceptibility to drought -
experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench**

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Abstract

Aims

We investigated the response of the perennial grass *Molinia caerulea* (L.) Moench to combined effects of fertilization (N, P) and drought events. We hypothesized that N fertilization increases, and drought decreases productivity, but that N addition strengthens negative effects caused by drought.

Methods

Within a full-factorial two-year greenhouse experiment we measured biomass productivity and allocation, tissue nutrient concentrations and nitrogen allocation patterns using ^{15}N as a tracer.

Results

N fertilization caused a strong increase in productivity, but effects of drought were almost insignificant. However, we found strongly interrelated, non-additive effects of fertilization and drought, expressed by a strong increase of necrotic tissue. Dead aboveground biomass showed the highest values for N and ^{15}N .

Conclusions

Accelerated productivity of aboveground tissue under N fertilization resulted in increased evaporative demands and thus higher drought susceptibility. In addition ^{15}N allocation patterns showed that fertilization-drought treatments disabled plants' control of their N allocation. *Molinia* was unable to withdraw leaf N during the dieback of aboveground tissue. Due to the lack of an adaptive strategy to the combined effects of fertilization and drought, increasing summer drought may weaken the competitive performance of species with traits comparable to those of *Molinia* in N-fertilized environments.

Introduction

Atmospheric nitrogen (N) deposition and climate change are the main drivers of biodiversity loss and affect ecosystem functioning on global and regional scales (Sala et al. 2000; Tylianakis et al. 2008). Atmospheric N deposition has risen since the beginning of industrialization, with an upward trend expected in the near future (Galloway et al. 2004). N loads affect ecosystem nutrient levels and cycles, the growth of plants and their competitive interactions, and are responsible for shifts in the species composition of many plant communities (Alonso et al. 2001; Bobbink et al. 1998; Britton et al. 2001; Marcos et al. 2003; Schmidt et al. 2004; Stevens et al. 2006; von Oheimb et al. 2010). Even low levels of chronic N inputs may have long-lasting impacts on ecosystems, as demonstrated by Clark and Tilman (2008) using the example of prairie grasslands. While airborne N loads have affected ecosystems in recent decades, impacts of climate change are predicted to increase in importance, particularly in the course of this century. The Intergovernmental Panel on Climate Change (IPCC 2007) predicts rising mean annual temperatures, changes in precipitation patterns and alterations of the frequency and magnitude of extreme weather events (e.g. summer drought). On the ecosystem level, these changes can alter primary productivity, carbon sequestration and nutrient cycling (Gorissen et al. 2004; Grime et al. 2000; Walther 2010). On the species level, shifts in climate may affect the performance and range of species, but also species interactions such as competition for light and nutrients (Andresen et al. 2010; Fotelli et al. 2005; Gorissen et al. 2004; Grime et al. 2000; Walther 2010).

Assessments and predictions of species responses, however, are often difficult due to largely unknown interrelations between simultaneously acting drivers of global change. It is conceivable, for example, that species responses to climate change could be both mitigated and strengthened by other global change drivers such as N deposition. Species and their environments, however, may face both climate shifts and increasing N loads in the course of this century (Baeten et al. 2010; Tylianakis et al. 2008). Results from grassland experiments have shown that the combination of different drivers can produce non-additive interrelated

effects. Barnard et al. (2006) found that nitrifying enzyme activity in grassland soils was not affected by elevated CO₂ levels, but increased as a result of N addition. However, if both treatments were combined, the positive effects of N addition were no longer visible. Ritchie (2000) showed that thermal conditions and N availability interacted to influence herbivore abundance. In addition, positive effects of N loads on herbivore abundance were most pronounced under high rainfall conditions (Boyer et al. 2003). Since such interactions are sometimes unexpected and may remain undetected if single factors are analyzed in isolation (Betson et al. 2007), studies aiming to predict species responses to global change may be more meaningful when these factors are analyzed in combination, for example by means of full-factorial experimental approaches.

Shifts in water availability (e.g. due to summer droughts) and increasing N supply may have a particularly strong impact on plant growth and competition. Studies on the effects of summer droughts often found a reduction in plant growth and productivity (Baeten et al. 2010; Damgaard et al. 2009; Peñuelas et al. 2004; Shah and Paulsen 2003). N additions, by contrast, stimulated plant growth, but also increased the plants' shoot-root ratios (Aerts et al. 1991; Boot 1989; Ericsson 1995; Thornton 1991). The combination of both factors may implicate interactive effects on plant growth and competition: Biomass allocation to the shoots enables plants to compete more effectively for aboveground resources (Aerts and Chapin III 2000; Goldberg 1990). Therefore, this strategy is beneficial to plants as long as the nutrient and water supply is sufficient. The risk of water shortage during drought events because of an increasing transpiring surface, however, may result in a trade-off in biomass allocation (Aerts and Bobbink 1999). Thus, improved N availability could strengthen adverse effects of drought when both factors act simultaneously (Betson et al. 2007).

In a study by Gordon et al. (1999), increasing N availability in combination with drought strengthened growth reductions (due to drought) of *Calluna vulgaris*, but not of *Pteridium aquilinum*. These results indicate that plant responses are species-specific, and the consideration of functional types may be helpful to detect general response patterns. MacGillivray et al. (1995) found that the plants ability to tolerate nutrient deficiency correlated

positively with the plants' resistance to extreme events such as frost or drought, but negatively with their resilience (i.e. speed of recovery). Hence, fast-growing species with high nutrient requirements and competitiveness tended to be more sensitive to drought, but may recover faster than plants characterized by a high nutrient stress tolerance. Such differences in drought sensitivity may mediate the competitive relationships between different functional plant types. Morecroft et al. (2004), for example, found a decrease in the percentage cover of perennial grasses as a result of drought in grassland ecosystems, while the cover of short-lived ruderal species increased.

For the prediction of potential changes in plant growth and competition as a result of environmental shifts it is, thus, crucial to understand and to quantify plant responses (in terms of their biomass and nutrient allocation) to combined effects of global change drivers (i.e. N deposition, increase of drought events) and in relation to functional types. In the present study, we investigated effects of simulated drought events and N deposition on the performance of a common European grass species in a full-factorial two-year pot experiment. We selected *Molinia caerulea* (L.) Moench (henceforth referred to as *Molinia*) as a focal species for several reasons. Firstly, *Molinia* is a fast-growing perennial grass, which has considerably increased in frequency and cover in various European habitats of high conservation value, for example in wet and dry heaths, but also in mires and moorlands (Aerts et al. 1991; Brys et al. 2005; Chambers et al. 1999; Diemont and Heil 1984; Falk et al. 2010). Thus, the analysis of *Molinia* responses may contribute to an understanding of mechanisms underlying the ongoing process of *Molinia* encroachment under current and prospective environmental shifts. Secondly, our findings may help to characterize and predict response patterns with regard to global change typical of perennial grasses (as an important functional type) which show high competitive vigour and expansiveness. Since high N additions may cause a shortage of phosphorus (P) (Verhoeven et al. 1996), a P-addition treatment was included in our experiment. Nutrient treatments were performed during two growing seasons, while the drought treatment started in the second year of the experiment. In this way, drought took effect on already fertilized plants, hence simulating possible impacts

of drought events on systems that have already undergone atmospheric inputs of N (i.e. drought will affect plant growth in addition to the already existing N deposition). Growth responses were measured in terms of biomass allocation and tissue N and P concentrations. Our analyses were complemented by a ^{15}N tracer experiment (second year) in order to determine N allocation patterns resulting from treatments. We hypothesize that (i) N addition increases shoot-root ratios of *Molinia*, (ii) drought treatments reduce above- and belowground growth, and (iii) N addition increases the species' susceptibility to drought (expressed by decreasing productivity).

Materials and methods

Experimental design

Molinia caerulea (L.) Moench seeds were collected in the Lüneburger Heide nature reserve (NW Germany) and transferred to germination dishes in a non-tempered greenhouse in September 2007. Seedlings emerged in the middle of March 2008 and were transferred to plant pots (12 x 12 x 12 cm³) that contained nutrient-poor sand (of 7 cm thickness) covered by a humus layer (of 4 cm thickness and using soil material collected from the humus horizons in the Lüneburger Heide nature reserve). 16 plant individuals were planted together in one pot (equidistantly in 4 rows and lines).

All pots were randomly assigned to a full-factorial combination of the following treatments: N addition, P addition, and drought (treatments are henceforth referred to as N, P, and D treatment, respectively). The following treatments were applied in the first year (2008): control, N, P and N+P (i.e. combination of N and P) using 30 pots per treatment. At the end of the 2008 growing season, 10 of the 30 pots per treatment were harvested (see description below). The following treatments were carried out in the second year (2009): control, N, P, N+P, D, N+D, P+D, N+P+D, using 10 pots respectively of the pots remaining from the corresponding treatments in 2008 (i.e. 10 pots per treatment and year). N-treated pots received 48 kg N ha⁻¹ yr⁻¹ (NH₄NO₃), P-treated pots received 4 kg P ha⁻¹ yr⁻¹ (Na₂HPO₄) and N+P-treated pots received a combination of both. Controls received deionised water only.

Nutrient solutions were applied weekly from May through the end of August over two growing seasons. Pots were kept in a greenhouse and their arrangement was changed monthly at random. All pots were watered regularly to prevent water stress, with the exception of pots receiving drought treatments. Drought treatments were carried out three times during the growing season (first week of June, first week of July, last week of July). Each drought period started after the weekly fertilizer addition and lasted for one week until the next fertilizer addition. During the growing season, D-treated pots received approximately 18 % less water than controls.

¹⁵N tracer additions

¹⁵N tracer additions were performed during the second growing season (i.e. 2009). ¹⁵N tracer was added three times to all pots. The ¹⁵N tracer addition was given at the June 2, June 30 and July 21, that is always one day before a drought period was initiated. Each pot received 0.1 mg ¹⁵NH₄¹⁵NO₃ (99.22 atom%) dissolved in 70 ml deionized water. The total amount of 0.3 mg ¹⁵NH₄¹⁵NO₃ was calculated to achieve a distinct ¹⁵N tracer signal in the plant biomass, but was too small to cause a fertilization effect in non-N treated pots (Friedrich et al. 2011).

Harvesting and chemical analyses

In 2008, 10 pots (of the 30 pots per treatment) were harvested at the beginning of September (when plants were still in a fresh and green status). A second harvest (of all remaining pots) took place after the second growing season in 2009. For both years we quantified aboveground and belowground biomass production and related biomass allocation patterns. We separated the aboveground biomass (leaves and flower stalks) into (i) living tissue, (ii) dead tissue, and (iii) basal internodes. In addition, we determined the shoot-root ratio, N and P biomass concentrations and the ¹⁵N tracer recovery to assess ¹⁵N allocation patterns (in 2009).

After harvest, biomass samples were dried at 80°C for 12 h and weighed. Subsequently, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany) or

ground with a mixer mill (MM 400, Retsch, Haan, Germany), and re-dried at 105 °C before weighing. N and ^{15}N concentrations were analyzed using a continuous flow elemental analyzer-isotopic ratio mass spectrometer (vario EI cube, Elementar, Hanau, Germany, coupled to an Isoprime IRMS, Isoprime Ltd., Cheadle Hulme, UK). For P determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550° C for three hours. Ash samples were dissolved in an HCl solution, which was evaporated to dryness. Residues were again dissolved in an HCl solution (Schlichting et al. 1995) and analysed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Calculation of ^{15}N recovery and ^{15}N allocation patterns

^{15}N tracer recovery in the plant parts (living and dead aboveground biomass, basal internodes and belowground biomass) was calculated as follows:

$$^{15}\text{N}_{\text{rec}} = N_{\text{tot}} * (\text{atom}\%^{15}\text{N}_{\text{labeled}} - \text{atom}\%^{15}\text{N}_{\text{ref}}) / (\text{atom}\%^{15}\text{N}_{\text{tracer}} - \text{atom}\%^{15}\text{N}_{\text{ref}}),$$

where $^{15}\text{N}_{\text{rec}}$ is the mass of ^{15}N tracer recovered in the plant tissue of a given plant part of labeled plants (mg N per plant), N_{tot} is the total mass of N in the plant tissue of labeled plants (mg N per plant), $\text{atom}\%^{15}\text{N}_{\text{labeled}}$ is the $\text{atom}\%^{15}\text{N}$ in the plant tissue of labeled plants, $\text{atom}\%^{15}\text{N}_{\text{ref}}$ is the $\text{atom}\%^{15}\text{N}$ in the plant tissue of non-labeled plants, and $\text{atom}\%^{15}\text{N}_{\text{tracer}}$ is the $\text{atom}\%^{15}\text{N}$ of the added ^{15}N tracer (modified equation according to Nadelhoffer et al. (2004)). We used $\text{atom}\%^{15}\text{N}$ of biomass samples from non-labeled, but N- and P-treated plants harvested in the first year as $\text{atom}\%^{15}\text{N}_{\text{ref}}$. In this way, we accounted for differences in natural ^{15}N abundances caused by N additions during the first year of the study. ^{15}N allocation to a given plant part was calculated as the percentage of ^{15}N recovered in the plant part relative to the total amount of ^{15}N recovered in the total plant.

Data evaluation and statistics

Treatment effects on biomass dry weights (dw) of the different plant parts, shoot-root ratios, tissue N and P concentrations and ^{15}N allocation were compared by means of one-way ANOVA with a Tukey's post hoc test ($P < 0.05$). Interrelated treatment effects (i.e. type of fertilization, drought) were tested using Generalized Linear Models (GLM). Each GLM included the main effects of N, P and drought as well as the first order interaction terms (N x P, N x D, P x D). Analyses were conducted with SPSS 17.0 (SPSS Inc., Chicago, IL).

Data evaluation for the first growing season was restricted to an analysis of shoot-root ratios as affected by treatments (using living plant material). All data in Figures and Tables are given on a per plant basis (with the exception of Figures referring to ^{15}N allocation (%) and shoot-root ratios). In order to evaluate nutrient limitation of plant growth, tissue nutrient concentrations were plotted as a function of nutrient contents following the approach of Timmer and Stone (1978). To this end, the total aboveground biomass per plant was multiplied by the tissue nutrient concentration (biomass means per plant) to calculate the total nutrient contents per plant. Trajectories of shifts in nutrient concentration and nutrient content as a result of fertilization were plotted in a nutrient content - nutrient concentration space. The trajectories indicate whether an increase in nutrient contents occurred because a nutrient was limiting (a shift into the sector between the vertical and the horizontal line, i.e. increases in both nutrient concentrations and biomass) or due to luxury consumption (shifts of values along the vertical line, i.e. increased nutrient concentrations without any gain in biomass).

Results

Treatment effects on biomass production and allocation

Aboveground biomass of *Molinia* increased by a factor of 5 and 6 as a result of N and N+P fertilization, respectively (Fig. 1a). This increase was apparent for all plant parts, of which internodes showed the highest increase (seven- and six-fold in the N and N+P treatment,

respectively; calculated from data in Table 1). P treatments had no effect on the aboveground biomass (Fig. 1a), but N x P interactions were significant for internodes and dead aboveground biomass (Table 1). D treatment alone slightly, but significantly reduced the aboveground biomass (Fig. 1a), but this reduction was insignificant if single plant parts are considered (i.e. internodes, living and dead aboveground biomass; significances for the respective plant parts not shown in Fig. 1). By contrast, drought in combination with N strongly reduced both total and living aboveground biomass in comparison to the N treatment (living biomass was 59 mg (dw) and 123 mg (dw) in the N+D and N treatment, respectively; Table 1, Fig. 1a). Correspondingly, dead aboveground biomass increased by a factor of 6 and 10 in N+D and N+P+D pots, respectively, in comparison to controls. N x D interactions were significant for all aboveground plant parts (Table 1).

Treatments had similar effects on the belowground biomass. This is reflected in a sixfold and sevenfold biomass increase resulting from N and N+P fertilization, respectively (Fig. 1b). D treatments had no significant effects on belowground biomass, and fertilization effects were reduced under drought (fourfold and threefold increase in the N+D and N+P+D treatments, respectively). Accordingly, N x D interactions were significant (Fig. 1b, Table 1). P addition had no effect on belowground biomass.

During the first growing season (2008), shoot-root ratios doubled as a result of N and N+P addition, but were unaffected by P (Fig. 2a). In 2009, shoot-root ratios decreased (in comparison to 2008), and differences caused by treatments in 2008 were balanced. Drought reduced shoot-root ratios in combination with N (i.e. N+D and N+P+D treatments; Fig. 2b), and this was mirrored by significant N x D interactions (Table 1).

Treatment effects on nutrient concentrations (2008 and 2009)

N fertilization increased N concentrations in combination with D treatments (i.e. N+D and N+P+D), but not in the N and N+P treatments (with the exception of internodes; Fig. 3). Increases were significant for all plant parts, but were highest for the internodes (two- and three- fold increase in the N+D and N+P+D treatment, respectively; Fig. 3c). Combined

effects of N and D treatments resulted in significant N x D interactions (Table 1). P fertilization had no effect on N concentrations.

P concentrations increased for all plant parts in the P and P+D treatments, but declined in the N and N+P treatments in comparison to controls (Table 1). In the N+P+D treatment, P concentrations increased in the living aboveground tissue and in the internodes, but decreased in the belowground biomass.

N accumulation was associated with increasing biomass in N and N+P treatments, while N concentrations increased only slightly (Fig. 4a). By contrast, P fertilization increased P concentrations, but biomass increased only when N was also added (Fig. 4b). In D-treated pots N fertilization caused an increase in both biomass and N concentrations (Fig. 4c). P fertilization combined with drought increased P concentrations, but did not affect biomass production (Fig. 4d). In summary, drought in combination with fertilization caused higher tissue nutrient concentrations and lower biomass increases.

Treatment effects on ¹⁵N allocation (2009)

Treatments clearly affected ¹⁵N allocation patterns to the different plant parts (Fig. 5), in which a mean 25% of added tracer was recovered.

¹⁵N allocation to the living aboveground biomass (Fig. 5a) was reduced in the N and N+P treatment, but increased in the P treatment relative to the control (in the latter, 35% of recovered tracer was found). In contrast, D treatment resulted in insignificant differences in allocation patterns when combined with fertilization (i.e. N+D, P+D, N+P+D).

In the dead aboveground biomass (Fig. 5b), ¹⁵N sequestration was particularly high in the N+D and N+P+D treatments, but differences were insignificant for all the other treatments.

In the internodes (Fig. 5c), ¹⁵N sequestration did not differ for the N, P, and N+P treatments, but significantly decreased when N and D treatments were combined (i.e. N+D, N+P+D).

¹⁵N allocation to the belowground biomass (Fig. 5d) was higher than for other plant parts (relative allocation of ¹⁵N was > 50%). ¹⁵N sequestration was highest in the N and N+P

treatment, but differences were insignificant for the D treatment in combination with fertilization (i.e. N+D, P+D, N+P+D).

N x D interactions were significant for all plant parts (Table 1). The same applied to N x P interactions, with the exception of ^{15}N allocation to belowground biomass.

Discussion

Treatment effects on biomass production and allocation

Biomass of *Molinia* increased due to N fertilization, accompanied by increasing shoot-root ratios in the first, but not in the second growing season (Figs. 1 and 2). Higher belowground allocation in the second year should have compensated for differences in shoot-root ratios found in the first year. We hypothesize that increasing belowground allocation in the second year was attributable to an age but not to a fertilization effect (Müller et al. 2000). This interpretation is supported by the finding that shoot-root ratios declined across treatments in 2009, and thus also in the controls, indicating a process independent of nutrient availability.

In contrast to N fertilization, D treatment (without fertilization) had only a slight effect on total productivity, and effects were insignificant for the respective plant parts (such as living aboveground biomass, internodes, and belowground biomass; Fig. 1). This indicates that the experimentally imposed drought was not enough to cause a strong growth decline. Response patterns of *Molinia* to drought in our experiment coincide with the species' performance within a wide range of (partly artificial) habitats with strong gradients in water supply, a finding attributable to the species' morphological and physiological plasticity (Aerts et al. 1991; Salim et al. 1988; Taylor et al. 2001). In addition, *Molinia* is characterized by comparatively low shoot-root ratios (about 0.5 in unfertilized environments; Aerts et al. 1991; Fig. 2) that may support the species' ability to satisfy its water demands even during periods of drought (Aerts and Chapin III 2000; Goldberg 1990). However, if N additions have positive, and D treatments only slight (or even insignificant) effects on biomass production, then a decrease in productivity (compared to N treatments) accompanied by a distinct increase of necrotic biomass (six- to ten-fold; Fig. 1a) is at first an unexpected response to the

combination of these treatments, and is indicative of non-additive interrelated effects (Barnard et al. 2006; Fig. 1). We hypothesize that growth responses to combined effects of N and D were the result of water shortage attributable to the higher aboveground productivity (following N fertilization) and thus increased water requirements of plants due to higher transpiration rates (van Heerwaarden et al. 2005). Thus, if N fertilization takes place in combination with drought, *Molinia* is unable to meet its evaporative demands, thus ultimately leading to leaf wilting and the formation of necrotic tissue (Brooks and Coulombe 2009; Gordon et al. 1999; Nilsen 1995). This process should affect N-limited plants in particular, since N inputs may then cause a disproportionate increase in leaf biomass, and hence an increased probability of water stress (Högberg et al. 1993). As a consequence, N fertilization may strengthen a plant's susceptibility to periods of drought in N-limited environments. This result is in agreement with the observations of Betson et al. (2007), who found an increasing susceptibility of *Pinus sylvestris* to drought events in forests exposed to moderate to high N deposition (using the trees' $\delta^{13}\text{C}$ foliage signature as a response variable). However, our results are inconsistent with those of Saneoka et al. (2004), who found that higher levels of N nutrition increased the drought tolerance of the grass *Agrostis palustris*.

In contrast to the morphological and physiological plasticity with which *Molinia* responds to environmental gradients (Thornton 1991), N-fertilized plants exhibited no adaptive strategy to D treatments in our experiment, for example by increased belowground productivity (Asseng et al. 1998; Fotelli et al. 2002), decreasing shoot-root ratios (Gonzalez-Dugo et al. 2010; Kahmen et al. 2005; Lösch 2001), or "compensation growth" that may counterbalance losses of photosynthetic active tissue (Ericsson 1995; Xu et al. 2009). This result may be partly ascribed to our experimental design, in which plants were fertilized in the first, and exposed to both fertilization and drought in the second year. Since *Molinia* is characterized by a highly efficient N (re)cycling and storage strategy (see discussion below), a high proportion of N stored in roots and internodes (in 2008) may have contributed to the fast production of aboveground tissue in spring 2009 (Thornton 1991), even before the first D treatment was applied. As a consequence, fertilized plants showed increased evaporative demands

(compared to controls) and thus higher drought susceptibility, despite their higher belowground allocation in the second year. This interpretation in turn supports the hypothesis that N fertilization of (primarily) N-limited plants may foster their productivity in the early growing season and thereby their water requirements in summer, increasing the probability of water shortage during drought events (Högberg et al. 1993).

Treatment-related nutrient concentrations and ^{15}N allocation

Both patterns of productivity and nutrient concentrations indicated that growth of *Molinia* was limited by N, a finding that is in agreement with studies by Aerts (1990), Thornton (1991), and Falk et al. (2010; Figs. 1, 3, and 4). This is reflected in insignificant differences in tissue N concentrations across treatments (Fig. 3; with the exception of N+D and N+P+D), since sequestered N contributed to a significant increase in productivity (Fig. 4). P, in turn, had no effect on productivity and thus was not a growth-limiting nutrient (Fig. 4). D treatments, by contrast, caused a lowered productivity of N-fertilized plants (i.e. N+D and N+P+D treatments). This is reflected in the higher tissue N concentrations (Figs. 3a, c), since N was partly accumulated in leaves and internodes due to the reduced biomass. We hypothesize that two mechanisms may have contributed to reduced productivity in N+D and N+P+D treatments. Firstly, plants closed their stomata and hence decreased their photosynthetic rate during the experimentally imposed drought (Gonzalez-Dugo et al. 2010; Lösch 2001; Shah and Paulsen 2003). Second, a high proportion of necrotic tissue may have weakened the growth vigour of plants due to a shortage of photosynthates, which in turn may appear when shoot-root ratios of about 0.2 are achieved (see Fig. 2c; Chapin III et al. 1987; Gordon et al. 1999; Shah and Paulsen 2003; Ward et al. 1999).

A striking finding, however, was that dead aboveground biomass showed the highest values for N and ^{15}N (Figs. 3b, 5b). This indicates that *Molinia* was unable to withdraw N during the drought-induced dieback of aboveground tissue. This is in contrast to experimental findings, which showed *Molinia* to have a highly efficient N-resorption strategy which, for example, enables plants to withdraw about 85% of N from senescing leaves (van Heerwaarden et al.

2005). Obviously, the formation of necrotic tissue occurred too fast to allow for an efficient resorption of leaf N. Thus, the combined effects of fertilization and drought disabled *Molinia*'s control of its N cycling and allocation. This interpretation is supported by the result that ^{15}N allocation patterns to aboveground and belowground biomass differed significantly between fertilization treatments and controls, but differences were insignificant when fertilization and drought were applied simultaneously (i.e. no "controlled" allocation; Fig. 5a, d). This response pattern was unexpected, since *Molinia* is known for its high (e.g. morphological) adaptability to environmental gradients and shifts (Aerts et al. 1991; Thornton 1991). Our results, by contrast, indicate that *Molinia* lacks an adaptive strategy to environmental conditions in which drought events follow fertilization. It is, thus, conceivable that climate shifts (such as increasing summer drought) in combination with airborne N loads may weaken the competitive performance of this grass species in its current habitats. Our findings support the hypothesis of MacGillivray et al. (1995) that plant traits which promote high productivity (in the case of *Molinia* the usage of stored and recycled N for accelerated productivity) lead to a correlated susceptibility to extreme events (such as drought). This in turn may cause shifts in the species composition of affected environments (Morecroft et al. (2004).

In conclusion the results of the present study demonstrate that combinations of N fertilization and drought may result in strongly interrelated, non-additive effects on plant growth. Results of treatment-related biomass and nutrient allocation patterns show that *Molinia* suffered severely from environmental conditions in which drought events followed N fertilization. Thus, despite its adaptability to a wide range of environmental conditions, the species lacks an adaptive strategy to combined effects of fertilization and drought. As a consequence, increasing summer drought (as currently predicted by climatic models) may weaken the species' competitiveness in N-fertilized environments (e.g. due to airborne N inputs). This may also apply to other perennial, fast-growing plant species with traits similar to those of *Molinia* such as the mobilization of stored N for a rapid increase in aboveground biomass during the early growing season.

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Table 1 Means and standard errors (in parentheses) of the plant parts analyzed (above alive: living aboveground biomass, above dead: dead aboveground biomass, internodes: biomass of basal internodes, below: belowground biomass; all in mg dry weight per plant (dw)), shoot-root ratios, tissue N and P concentrations (in mg g⁻¹ dw) and ¹⁵N allocation (in % of total recovered ¹⁵N) in relation to treatments (C: control; N: nitrogen; P: phosphorus; N+P: nitrogen and phosphorus; D: drought; N+D: nitrogen and drought; P+D: phosphorus and drought; N+P+D: nitrogen, phosphorus and drought). Results of the GLM analyses with interactions of nitrogen (N), phosphorus (P) and drought (D) are given where significant: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Beitrag V

	C	N	P	N+P	D	N+D	P+D	N+P+D	Interactions
<i>Biomass (mg dw)</i>									
above alive	25.5 (0.8)	123.2 (4.5)	27.7 (1.4)	147.5 (4.3)	20.2 (1.2)	59.0 (9.6)	22.2 (0.9)	38.6 (9.8)	N x D*** P x D*
above dead	17.0 (1.0)	41.0 (2.1)	9.5 (0.9)	55.5 (2.7)	10.0 (0.6)	107.5 (13.5)	12.6 (0.7)	170.7 (6.3)	N x P*** N x D*** P x D***
internodes	13.9 (0.6)	91.0 (3.2)	13.0 (0.6)	81.3 (4.0)	12.8 (0.7)	34.9 (7.5)	12.6 (0.5)	16.9 (6.1)	N x P* N x D***
below	107.2 (8.9)	596.5 (37.5)	100.8 (7.6)	693.8 (55.8)	108.7 (9.1)	404.7 (29.7)	94.0 (4.2)	317.1 (21.2)	N x D***
shoot-root ratio	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)	0.2 (0.0)	0.4 (0.0)	0.2 (0.0)	N x P* N x D**
<i>N concentration (mg g⁻¹ dw)</i>									
above alive	10.9 (0.2)	12.7 (0.5)	11.8 (0.3)	10.9 (0.3)	12.4 (0.3)	18.0 (1.5)	12.0 (0.3)	21.5 (1.5)	N x D***
above dead	3.7 (0.2)	3.1 (0.1)	3.6 (0.1)	3.8 (0.1)	3.4 (0.1)	7.4 (0.7)	3.8 (0.1)	9.1 (0.6)	N x P* N x D***
internodes	3.7 (0.1)	4.8 (0.1)	4.0 (0.2)	5.0 (0.1)	4.5 (0.2)	8.4 (0.8)	5.5 (0.3)	11.7 (1.4)	N x D*
below	4.3 (0.3)	5.3 (0.1)	5.0 (0.2)	5.2 (0.2)	4.8 (0.2)	5.9 (0.2)	5.1 (0.2)	6.3 (0.4)	
<i>P concentration (mg g⁻¹ dw)</i>									
above alive	1.2 (0.1)	0.5 (0.0)	1.6 (0.1)	0.7 (0.1)	1.3 (0.1)	1.0 (0.2)	1.8 (0.2)	2.2 (0.3)	N x D*** P x D*
above dead	0.5 (0.0)	0.1 (0.0)	0.7 (0.0)	0.2 (0.0)	0.4 (0.0)	0.3 (0.0)	0.8 (0.1)	0.5 (0.1)	N x D**
internodes	1.3 (0.1)	0.4 (0.0)	1.5 (0.1)	0.7 (0.0)	1.3 (0.1)	1.0 (0.2)	1.6 (0.1)	2.3 (0.3)	N x P* N x D*** P x D**
below	0.9 (0.0)	0.3 (0.0)	1.1 (0.1)	0.5 (0.0)	1.0 (0.1)	0.3 (0.0)	1.3 (0.1)	0.5 (0.0)	
<i>¹⁵N allocation (%)</i>									
above alive	35.1 (1.8)	28.0 (1.1)	41.2 (1.5)	25.6 (1.0)	31.1 (0.9)	24.7 (2.6)	30.6 (0.8)	21.3 (3.5)	N x P* N x D***
above dead	3.2 (0.3)	1.4 (0.2)	1.7 (0.2)	2.4 (0.2)	1.0 (0.1)	11.7 (2.8)	2.9 (0.3)	22.9 (2.9)	N x P* N x D*** P x D**
internodes	7.4 (0.4)	9.5 (0.2)	7.7 (0.4)	8.7 (0.7)	8.2 (0.4)	6.4 (0.9)	9.9 (0.5)	3.7 (0.8)	N x P*** N x D***
below	54.3 (2.4)	61.1 (0.8)	49.5 (1.6)	62.9 (1.3)	59.8 (1.0)	57.3 (2.4)	55.8 (0.6)	52.1 (2.9)	N x D***

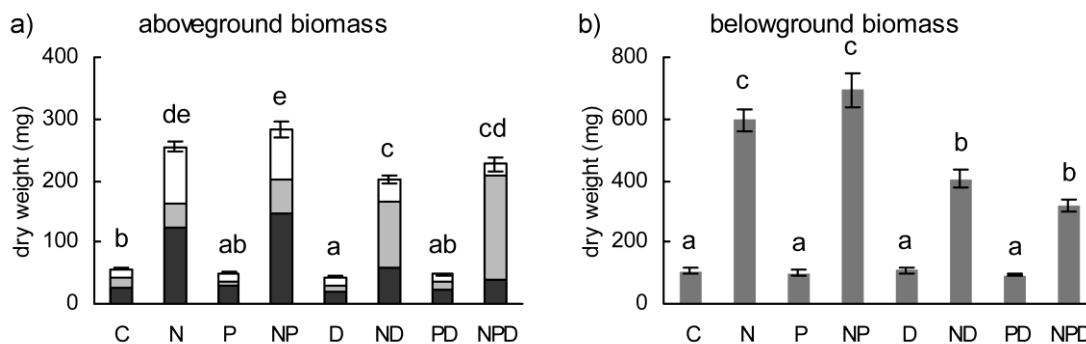


Fig. 1 Effects of fertilization and drought treatments on biomass dry weights (in mg; for abbreviations see legend of Tab. 1). Data refer to plant individuals and show means and standard errors (error bars) of aboveground biomass (a) and belowground biomass (b) harvested in 2009. Aboveground biomass is separated into living biomass (black), dead biomass (grey) and basal internodes (white). Different letters indicate significant differences found for the total aboveground biomass according to one-way ANOVA ($P < 0.05$)

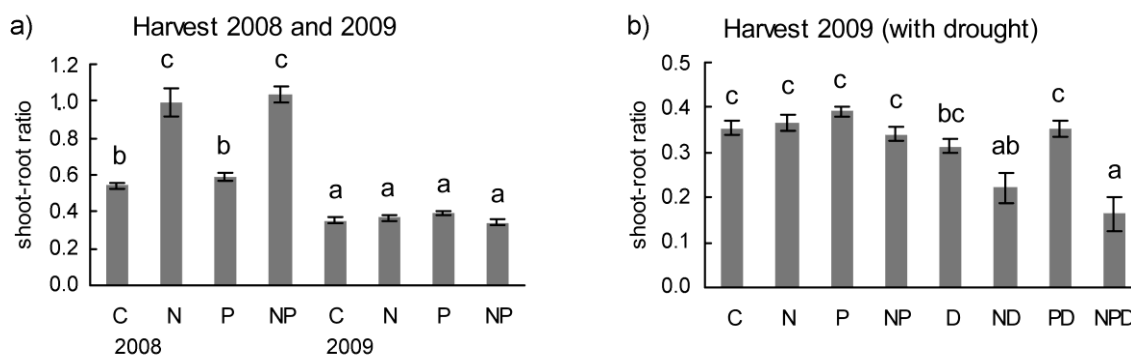


Fig. 2 Means and standard errors (error bars) of shoot-root ratios of biomass harvests after fertilization treatments in 2008 and 2009 (a), and biomass harvest after the combination of fertilization and drought treatments in 2009 (b); for abbreviations see legend of Tab. 1. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA

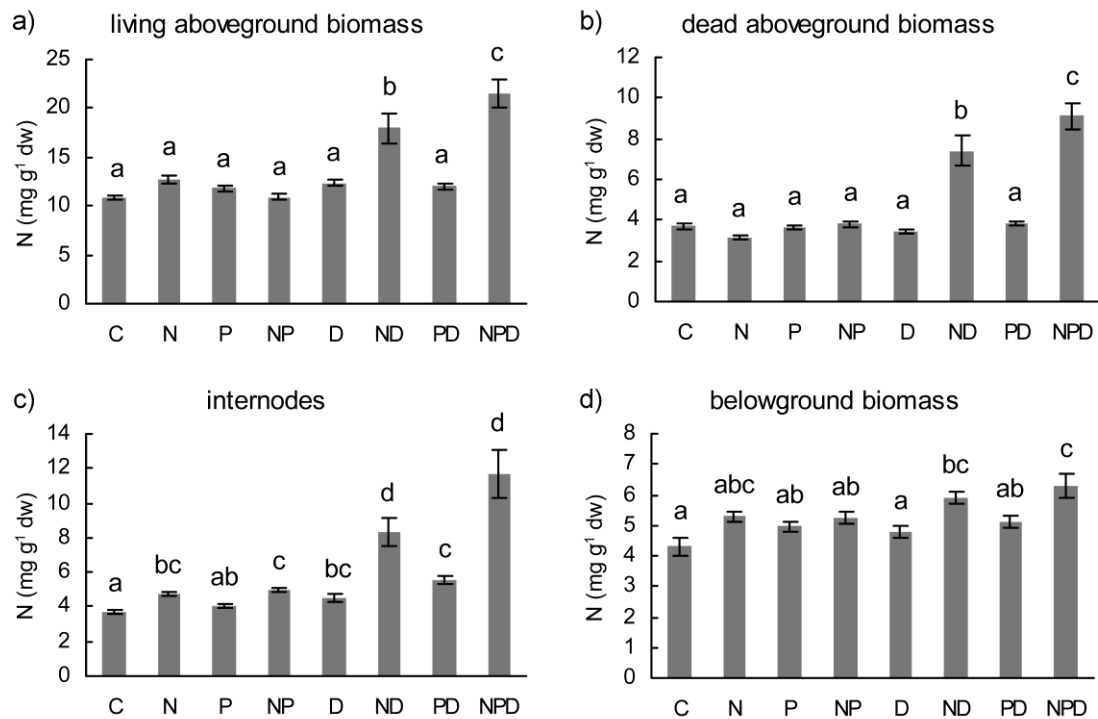


Fig. 3 Effects of fertilization and drought treatments on tissue N concentrations (in mg N g⁻¹ dw). Data refer to plant individuals and show means and standard errors (error bars) of: living aboveground biomass (a), dead aboveground biomass (b), basal internodes (c), and belowground biomass (d); for abbreviations see legend of Tab. 1. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA

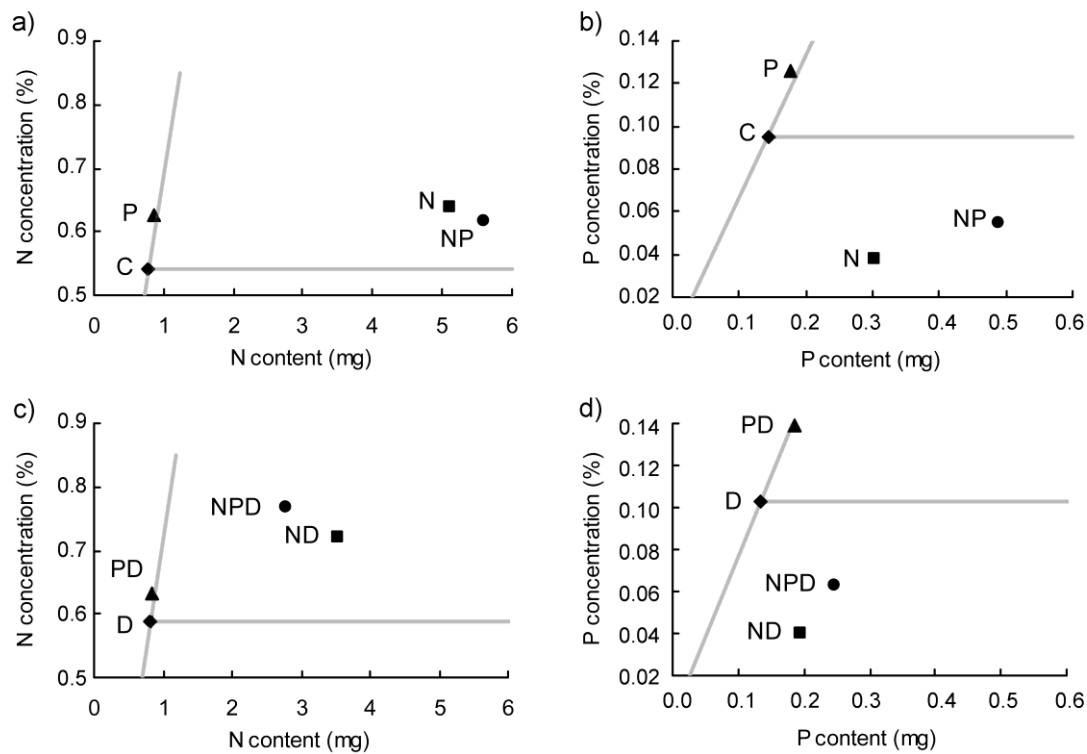


Fig. 4 Nutrient concentrations (%) vs. nutrient contents (mg) of total biomass per plant individual for nitrogen (N) and phosphorus (P) in regularly watered (a: N; b: P) and drought (D) treated pots (c: N; d: P). Shifts of values along the vertical line indicate increased nutrient accumulation without gains in biomass (luxury consumption), while shifts of values along the horizontal line indicate increased nutrient contents and biomass without changes in concentrations. A shift into the sector between both lines denotes increases in both nutrient concentrations and biomass, indicating that the initial nutrient level was growth limiting. A shift into the sector below the horizontal line indicates that the nutrient concentration has been diluted due to additional growth

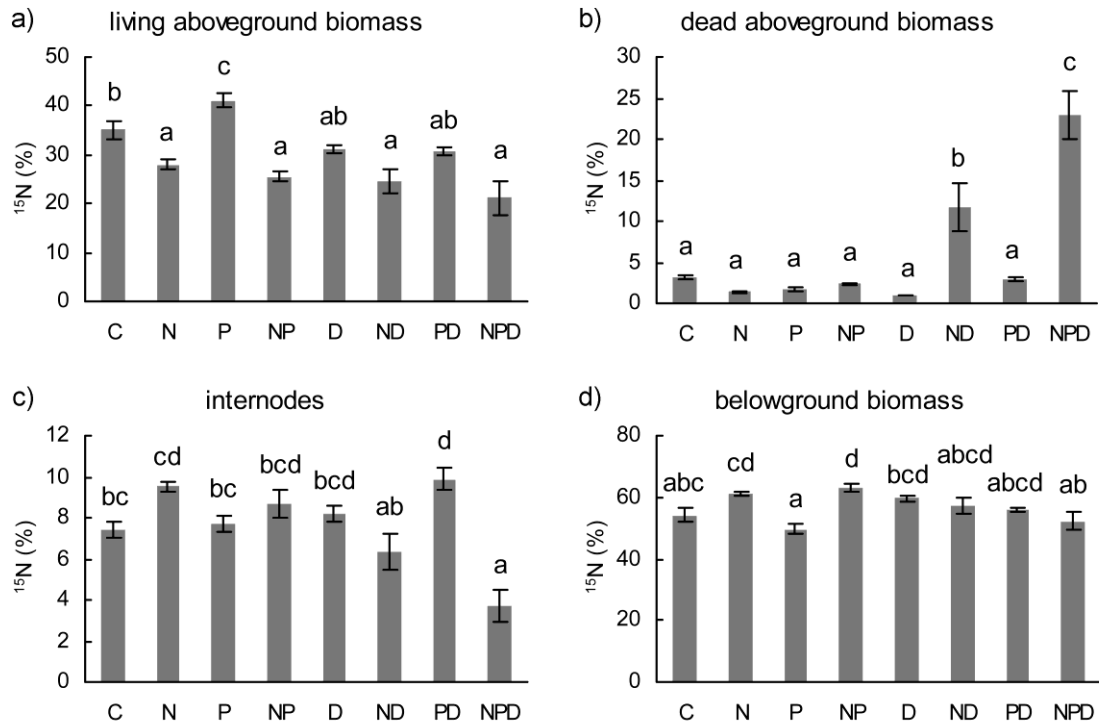


Fig. 5 Effects of fertilization and drought treatments on ^{15}N allocation (in % of total recovered ^{15}N per plant). Data show means and standard errors (error bars) of: living aboveground biomass (a), dead aboveground biomass (b), basal internodes (c), and belowground biomass (d); for abbreviations see legend of Tab. 1. Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA

Publikationsliste

- Friedrich U., von Oheimb G., Kriebitzsch W.-U., Schleßelmann K., Weber M. S., Härdtle W. (*under review*) Nitrogen deposition increases susceptibility to drought – experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench.
- Härdtle W., Niemeyer T., Assmann T., Aulinger A., Baiboks S., Friedrich U., Neuwirth B., Pfister L., Quante M., Ries C., Schuldt A., Simon N., von Oheimb G. (*submitted*) Long-term responses of *Fagus sylvatica* L. to environmental shifts in central Europe reflected in tree-ring width and stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$).
- Friedrich U., von Oheimb G., Dzedek C., Kriebitzsch W.-U., Selbmann K., Härdtle W. (*in press*) Mechanisms of *Molinia caerulea* encroachment in dry heathland ecosystems with chronic nitrogen inputs. *Environmental Pollution*, doi:10.1016/j.envpol.2011.08.010.
- Friedrich U., Falk K., Bahlmann E., Marquardt T., Meyer H., Niemeyer T., Schemmel S., von Oheimb G., Härdtle W. (2011) Fate of airborne nitrogen in heathland ecosystems: a ^{15}N tracer study. *Global Change Biology*, 17, 1549-1559.
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- von Oheimb G., Power S.A., Falk K., Friedrich U., Mohamed A., Krug A., Boschatzke N., Härdtle W. (2010) N:P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands. *Ecosystems*, 13, 317-327.

Mitwirkung bei wissenschaftlichen Referaten und Posterpräsentationen

- Friedrich U., von Oheimb G., Härdtle W. (2011) Fate of airborne nitrogen in heathland ecosystems – results of a ^{15}N tracer study. 12th European Heathland Workshop, Spain – Portugal.
- von Oheimb G., Friedrich U., Dzedek C., Härdtle W. (2011) Competitive superiority of *Molinia caerulea* in *Calluna*-heathlands under high N availability. 12th European Heathland Workshop, Spain – Portugal.

- Härdtle W., Falk K., Friedrich U., Meyer-Grünefeldt M., Merkle K., von Oheimb G. (2009) *Molinia caerulea* responses to N and P fertilisation in dry heathland ecosystems. 11th European Heathland Workshop, Falmouth, Cornwall, UK.
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- Friedrich U., Marquardt B., von Oheimb G., Härdtle W. (2007) Plant species composition in beech forest ecosystems: The importance of nitrogen and phosphorus supply. Kolloquiumsvortrag, Institut für Ökologie und Umweltchemie, Leuphana Universität Lüneburg, Lüneburg, Deutschland.

Lebenslauf

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Erklärung

Ich versichere, dass ich die eingereichte Dissertation „Impact of nitrogen deposition on the functioning of heathland ecosystems“ selbständig und ohne unerlaubte Hilfsmittel verfasst habe. Anderer als der von mir angegebenen Hilfsmittel und Schriften habe ich mich nicht bedient. Alle wörtlich und sinngemäß den Schriften anderer Autorinnen oder Autoren entnommenen Stellen habe ich kenntlich gemacht.

Hiermit erkläre ich, dass die Abhandlung in der gegenwärtigen oder einer anderen Fassung noch keinem anderen Fachbereich vorgelegen hat. Den Inhalt der Dissertation habe ich noch nicht im Rahmen einer Diplom- oder anderen Prüfungsarbeit verwendet.

Lüneburg, 24. August 2011

Uta Friedrich