Vegetation development in intact and restored base-rich sand ecosystems under different abiotic and biotic influences

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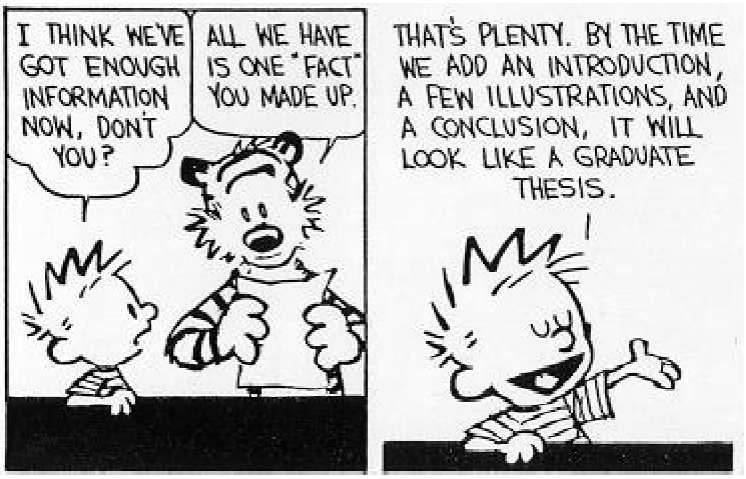
Freund, L., Eichberg, C., Retta, I. & Schwabe, A.: Seed addition via epizoochorous dispersal in restoration practice: an experimental approach mimicking the colonization of bare-soil patches. – 56th Annual Symposium of the International Association for Vegetation Science, 26-30 June 2013, Tartu, Estonia.

Freund, L. & Storm, C.: Nutrient additions in pioneer stages of sandy grassland: impact on phytomass production and nutrient contents. – Open Landscapes, 29 Sept - 3 Oct 2013, Hildesheim.

Storm, C. & Freund, L.: A long-term nutrient addition experiment in temperate sandy grassland: nutrient concentrations, phytomass production and community response. – 57th Annual Symposium of the International Association for Vegetation Science, 1-5 Sept 2014, Perth, Western Australia.



´Calvin and Hobbs´ by Bill Watterson

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Abbreviations

BB	Braun-Blanquet
BSC	Biological Soil Crust
D	Donor Site
DCA	Detrended Correspondence Analysis
EIV	Ellenberg Indicator Value
GP	Grid Plot
ND	Naturdenkmal (nature monument)
NMDS	Non-metric Multidimensional Scaling
NSG	Naturschutzgebiet (nature reserve)
PFT	Plant Functional Type
RS	Restoration Site
S1	Site 1 (Apfelbachdüne)
S2	Site 2 (Apfelbachdüne)
SADIE	Spatial Analysis by Distance Indices
SD	Standard Deviation
SE	Standard Error
TSR_{qual}	qualitative Target Species Ratio
TSR_{quant}	quantitative Target Species Ratio

Zusammenfassung

Sandökosysteme in Mitteleuropa sind durch Fragmentierung, Nutzungsaufgabe oder -intensivierung in ihrem Vorkommen stark gefährdet. Daher ist neben der Erhaltung bestehender Sandhabitate auch die Restitution degradierter oder zerstörter Flächen von großer Bedeutung. Dazu muss vor Beginn einer Restitutionsmaßnahme zunächst festgelegt werden, welche Pflanzengesellschaft Zielpunkt der Maßnahme sein soll; dabei sollten intakte Habitate als Referenzflächen herangezogen werden. Neben Kenntnissen über die gemeinschaftsbildenden Pflanzenarten (in speziellen Studien auch Tierarten), sollte auch bekannt sein, in welchen Konzentrationen Bodennährstoffe vorkommen und welcher Nährstoff in dem entsprechenden Ökosystem limitierend wirkt. Auf dieser Basis können entsprechende Maßnahmen geplant werden, um die Restitutionsfläche den abiotischen Bedürfnissen der Zielgesellschaft anzupassen. Als nächster Schritt ist zumeist ein gezieltes Einbringen von Diasporen der erwünschten Pflanzenarten auf die Restitutionsfläche notwendig, da die Zielarten meist eine geringe Ausbreitungskapazität besitzen und ohne Hilfe die Fläche gar nicht oder nur sehr langsam besiedeln könnten. Schließlich ist für naturnahe, vom Menschen geprägte Ökosysteme ein nachfolgendes Management nötig, um die Zielgesellschaft zu fördern und zu erhalten bzw. die Sukzession in Richtung monodominanter Grasbestände mit geringem Naturschutzwert zu verhindern.

Die drei Aspekte der Restitution, nämlich Abiotik, Biotik und Management, wurden in einem großflächigen Restitutionsvorhaben auf der ´Apfelbachdüne´ nördlich von Darmstadt (Weiterstadt, Landkreis Darmstadt-Dieburg) untersucht (**Kapitel 2**). Die auf einem ehemaligen Acker gelegene Restitutionsfläche entstand durch die Aufschüttung von Tiefensand, was der Nährstoffreduktion dienen sollte. Durch die Aufschüttung zweier Sandqualitäten (sehr nährstoffarm bzw. etwas phosphatreicher) in einer ´side-by-side´ Anordnung sollte der Einfluss der Sandqualität auf die Vegetationsentwicklung untersucht werden. Im Weiteren wurden die Ansätze ´Minimalinokulation´ und ´Beweidung mit Eseln´ untersucht, wozu auf diesen zwei Flächen je 16 Plots systematisch angeordnet wurden; die Verteilung der Behandlungen auf die Plots erfolgte randomisiert. Die Minimalinokulation wurde angewendet um zu erproben, ob kleinflächige ´Inseln´ mit Rechgut einer Donorfläche (etwa 5-7 % der Gesamtfläche) zur großflächigen Entwicklung von Sandtrockenrasen ausreichen. Zusätzlich wurden die Diasporen-Ausbreitungsvektoren ´Wind´ und ´Esel´ beprobt.

In Bezug auf die Sandqualität zeigte sich, dass diese eine große Rolle bei der Vegetationsentwicklung spielt, auf der P-reicheren Fläche kamen deutlich mehr Arten (auch Zielarten) bei gleichzeitig höherer Deckung vor als auf der nährstoffarmen Fläche. Allerdings konnte hierbei der Einfluss von P nicht sicher nachgewiesen werden, da das P-reichere Substrat durch oberirdische Lagerung vermutlich mit Diasporen verunreinigt worden war. Durch die Ausbringung des Rechguts konnten fast alle auf der Donorfläche nachgewiesenen Arten, zumeist Zielarten, auf die Restitutionsflächen transferiert werden, wodurch Zielarten auf den inokulierten Plots in Anzahl und Deckung überwogen. Beweidung führte zu einer Abnahme der Zielartendeckung inokulierter Plots, vornehmlich durch die Reduktion der Moos-Zielart *Tortula ruraliformis*. Nicht-inokulierte Plots und die zusätzlich untersuchten Rasterpunkte wurden von Ruderal- und Nicht-Zielarten dominiert, jedoch nahm die Anzahl von Zielarten während des vierjährigen Untersuchungszeitraums auf diesen Plots zu. Die Entfernung zwischen inokulierten Flächen und Rasterpunkten hatte kaum Effekte auf den Anteil von Zielarten oder deren Deckung. Im Vergleich mit Referenzflächen konnte in einer Ordination (DCA) eine Annäherung der inokulierten Plots an die Rechgut-Donorfläche und an eine bereits vor einigen Jahren restituierte Fläche beobachtet werden. Auch bei den nicht-inokulierten Plots deutete sich eine Entwicklung in diese Richtung an.

Von den untersuchten Ausbreitungsvektoren zeigte sich der Diasporenniederschlag deutlich von Nicht-Zielarten dominiert, insgesamt konnten nur sieben Zielarten mit wenigen Diasporen nachgewiesen werden. Die Epizoochorie-Proben ergaben eine saisonale Veränderung der transportierten Diasporen. Zum ersten Probennahmetermin in Juni wurden sowohl relativ viele Zielarten wie auch Diasporen von Zielarten erfasst (besonders *Medicago minima*). An den folgenden zwei Probennahmeterminen (Aug., Sept.) wurden vornehmlich Nicht-Zielarten erfasst. In den nur zu einem Zeitpunkt genommenen Endozoochorie-Proben konnten, wie schon bei der Epizoochorie, im Juni über die Hälfte der Arten und Keimlinge den Zielarten zugeordnet werden. Sowohl bei Epi- wie auch bei Endozoochorie wurden insgesamt nur relativ wenige Arten erfasst; es gab eine geringe Artenüberschneidung.

Neben dem Diasporentransport hatte die Beweidung mit Eseln bislang vor allem strukturelle Folgen. Besonders auf der nährstoffarmen Fläche kam es durch die Beweidung zu einem gleichbleibend hohen Offenbodenanteil, die Entwicklung einer Moosschicht wurde größtenteils verhindert. Auch die mit der Inokulation eingebrachte Moosschicht wurde dabei stark reduziert. Ein Zurückdrängen von Ruderalarten konnte bislang genauso wenig beobachtet werden wie eine Förderung von Zielarten.

Die Ergebnisse machen deutlich, dass für Restitutionsvorhaben dieser Art zwar auch Sand geringerer Qualität zur Aufschüttung genutzt werden kann, aber mit einer ruderal geprägten Entwicklung und höherem Pflegeaufwand gerechnet werden muss. Eine kurzzeitige oberirdische Lagerung sollte aufgrund der Gefahr einer Kontamination mit Diasporen und nährstoffreicherem Substrat vermieden werden. Durch Minimal-Inokulation können Zielarten erfolgreich auf Restitutionsflächen eingebracht werden und im Folgenden als Besiedlungs-Initiatoren der Gesamtfläche fungieren. Allerdings muss bei dieser Art der Restitution von einer langsamen Besiedlung ausgegangen werden. Weidetiere können zur Ausbreitung von Zielarten beitragen, sofern die Beweidung mit dem Zeitpunkt der Samenreife der Zielarten abgestimmt wird. Außerdem sollte die Beweidung den Verhältnissen der Fläche angepasst sein, um zu hohen (oder zu niedrigen) Weidedruck zu verhindern. Beweidung sollte durch die Erhaltung von Dynamik das langfristige Bestehen von Pioniervegetation sichern können.

Der tatsächliche Effekt von Epizoochorie – genauer die nachfolgende Etablierung der ausgebreiteten Pflanzenarten – wird in **Kapitel 3** behandelt. In einem früheren Experiment wurden Diasporen von ausgewählten Arten manuell auf dem Fell eines Schafes ausgebracht, welches anschließend einige Zeit auf offenen Sandflächen verbrachte. Die sich aus den epizoochor eingebrachten Diasporen entwickelnden Pflanzen wurden über einen Zeitraum von sechs Jahren beobachtet. Von den zehn untersuchten Arten konnten sich alle mit Ausnahme von *Jasione montana* auf mindestens einer der drei Flächen ansiedeln. Mehrjährige Arten blieben in ihrer Individuenzahl konstant oder sie nahm zu, wohingegen die einjährigen Arten keine einheitliche Entwicklung ihrer Populationen zeigten. Die zusätzlich untersuchten räumlichen Verteilungsmuster waren zumeist ´aggregiert´, bei mehrjährigen Arten blieben die Muster im Gegensatz zu einjährigen Arten über die untersuchten Jahre relativ ähnlich. Die sich auf den Epizoochorie-Flächen ansiedelnde Pflanzengemeinschaft wurde auch mit Blick auf die umgebende Vegetation und ein nahes Naturschutzgebiet analysiert. In einer Ordination (NMDS) konnte gezeigt werden, dass sich die Flächen in Richtung der Umgebungsvegetation entwickelten. In Bezug auf die Zielarten entsprachen die Flächen zwar mengenmäßig den im Naturschutzgebiet ermittelten, jedoch war die Zielarten-Deckung deutlich geringer und eine Reihe typischer Sandrasenarten fehlte noch in der Vegetation.

Mit diesem Experiment konnte gezeigt werden, dass sich mithilfe von Epizoochorie ausgebreitete Arten zumindest bei Vorhandensein von Offenboden ansiedeln können. Weidevieh könnte somit bei entsprechender Weideführung von Ziel- zu Restitutionsflächen das Einbringen von Zielarten ermöglichen.

Wiederum auf der Restitutionsfläche ´Apfelbachdüne´ angesiedelt, befasst sich **Kapitel 4** mit der Frage, ob bzw. wie sich die Einbringung stabiler biologischer Krusten auf die Entwicklung und Artenzusammensetzung neu entstehender Krusten auf initialem Substrat auswirkt. Dazu wurden insgesamt 16 Quadrate biologischer Krusten (à 11 cm x 11 cm) auf die nährstoffarme Fläche der Apfelbachdüne transplantiert; die Krusten stammen aus dem Koelerion glaucae-Bereich des ´Ehemaligen August Euler Flugplatzes´. Ein Vergleich der Artenzusammensetzung dieser Krustentransplantate mit jener von initialen Krusten in drei Entfernungen zum Transplantat und in Kontrollflächen über einen Zeitraum von drei Jahren sollte klären, ob Effekte distanz- und/oder zeitabhängig sind. Die Entwicklung der initialen Krusten wurde auch mithilfe von Chlorophyll *a*-Messungen verfolgt.

Bereits nach einem Jahr konnte eine initiale Kruste auf den Untersuchungsflächen nachgewiesen werden, die sich in ihrer Artenzusammensetzung kaum von den transplantierten Krusten unterschied. Da dies auch für die Kontrollflächen galt, wie eine Ordination (DCA) und Sørensen Indices deutlich machen, scheint der Einfluss der Krustentransplantate auf die Artenzusammensetzung eher gering zu sein. Allerdings blieben die Arten- und Taxazahlen sowie die Taxazahl von Cyanobakterien bis zum Ende der Untersuchungen in den drei Distanzen bzw. der Kontrolle deutlich geringer als in den Transplantaten, bei der Taxazahl von Grünalgen war dies jahresabhängig. Im Weiteren konnten distanz- und zeitabhängige Effekte der Transplantate auf die Gesamt- und Cyanobakterien-Taxazahl nachgewiesen werden. Dies gilt auch für die Sand-typischen, mit den Krustentransplantaten eingebrachten Moose, welche sich auf der Restitutionsfläche etablieren konnten. Bezüglich der Chlorophyll *a*-Gehalte konnte eine Zunahme vom ersten zum zweiten Jahr in der sich entwickelnden Kruste beobachtet werden, im dritten Jahr sank der Gehalt allerdings wieder etwas.

Zusammenfassend kann aus diesem Versuch geschlossen werden, dass sich die Einbringung von Krustentransplantaten bei verschiedenen Taxa unterschiedlich auswirkt. Die Etablierung und Artenzusammensetzung von Cyanobakterien und eukaryotischen Algen wurde während der primären Sukzession kaum von transplantierten Krusten beeinflusst; die Besiedlung der Fläche erfolgte höchstwahrscheinlich durch die Luft. Dagegen kann für Moose die Einbringung von Krustentransplantaten als Besiedlungs-Initiator wirken.

In **Kapitel 5** wurden die Auswirkungen von Nährstoffen auf ein intaktes Sandökosystem untersucht. Im Jahr 2000 wurde ein Nährstoffapplikations-Experiment in fünffach repliziertem und randomisiertem Blockdesign im Bereich von Koelerion glaucae Pioniervegetation auf dem ´Ehemaligen August-Euler-Flugplatz von Darmstadt´ gestartet. Die

acht Behandlungen umfassten neben einer Kontrolle die Gabe von Kohlenstoffquellen zur Stickstoffimmobilisierung (C), Phosphor (P), niedrig (n) und hoch dosierten Stickstoff (N), sowie Kombinationen von Stickstoff mit Mikro- und Makronährstoffen (NP, NPK, NPKM). Neben der Untersuchung der Vegetationsentwicklung lag der Fokus besonders auf der Auswertung von Phytomasse- und Nährstoffdaten verschiedener funktioneller Pflanzengruppen des Versuchszeitraums (bis mindestens 2010).

In Bezug auf die Phytomassegewichte zeigte sich, dass die ober- und unterirdische Phytomasse von Phanerogamen sowie die Streu auf stickstoffreich gedüngten Flächen zunahmen. Phosphat hatte keine Auswirkung auf die Phytomasse. Dagegen wurde die Phytomasseproduktion von Kryptogamen eher durch stickstoffarme bzw. -freie Düngung gefördert. Die Zunahme der Phanerogamen-Phytomasse unter N-Düngung macht deutlich, dass dieses Ökosystem stickstofflimitiert ist. In Bezug auf die Nährstoffkonzentrationen in den Pflanzengruppen zeigte sich, dass stickstoffreiche Düngung bei allen Gruppen zu einer Zunahme der N Konzentration führte. Gleiches gilt für die P Konzentration bei P Düngung, jedoch war der Effekt der P-Zunahme in der Phytomasse deutlicher ausgeprägt als bei N. Aus diesen Werten ließ sich das Verhältnis von Stickstoff zu Phosphat (N:P ratio) berechnen. Hierbei konnte gezeigt werden, dass auch in diesem Ökosystem die Verwendung des N:P Verhältnisses zur Ermittlung des limitierenden Nährstoffs möglich ist. Selbst bei Zufuhr von Stickstoff wurden Werte unter 14 ermittelt, was für Stickstofflimitierung spricht und sich mit den Ergebnissen der Phytomasseuntersuchung deckt. Auch die Anwendung von Ellenberg-Zeigerwerten, um Änderungen in den Standortverhältnissen abzubilden, zeigte sich hier als praktikabel. Besonders der Stickstoff-Zeigerwert nahm mit starker Stickstoffdüngung zu, gleiches gilt für den Feuchte-Zeigerwert, wohingegen der Licht-Zeigerwert mit Stickstoffdüngung abnahm. Zum Schluss konnte noch gezeigt werden, dass die schon zuvor beobachtete Trennung von Flächen ohne bzw. mit nur geringer N-Düngung und solchen mit hoher N-Düngung in zwei Sukzessionslinien weiterschreitet und mit den Jahren noch ausgeprägter wurde. Wiederum entwickelte sich die P-Behandlung nicht deutlich anders als die Kontrolle.

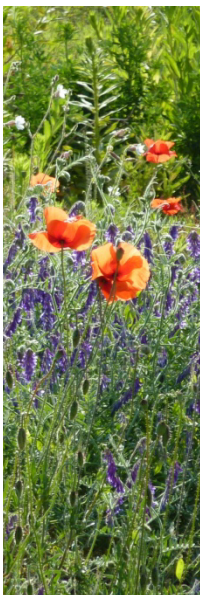
Aus den Ergebnissen kann geschlossen werden, dass zumindest geringe Stickstoffmengen, wie sie z.B. durch Deposition aus der Luft erfolgen, keine deutlichen Auswirkungen auf das Bestehen dieses Ökosystems haben. Erst bei sehr hohen N-Einträgen werden negative Auswirkungen auf die Vegetation und ihre Entwicklung deutlich. Durch Verschiebungen der Artenzusammensetzung und Steigerung der Produktivität können die typischen Pionierarten offener Standorte in ihrem Vorkommen gefährdet werden.

Eine Betrachtung aller Ergebnisse macht deutlich, dass das abgestimmte Zusammenspiel von Abiotik, Biotik und Management wichtig für erfolgreiche Restitutionsvorhaben ist. Mithilfe des Nährstoffapplikations-Experiments konnte Stickstoff als limitierender Faktor im hier untersuchten Sandökosystem, welches auch den Zielpunkt der Restitutionsvorhaben darstellt, nachgewiesen werden. Durch die Aufschüttung von Tiefensand wurden auf den untersuchten Restitutionsflächen geringe Stickstoffgehalte im Boden erreicht. Es konnte gezeigt werden, dass erstens das Einbringen von (Ziel-)Arten auf Restitutionsflächen (mittels Rechgut einer Leitbildfläche), zweitens Krustentransplantationen (transplantierte Moose) und drittens epizoochor durch Schafe eingetragene Diasporen erfolgreiche Maßnahmen sind, um die Diasporenlimitierung zu überwinden, wohingegen die Bildung von Grünalgen-/ Cyanobakterien-Krusten spontan aus der Luft stattfinden kann. Bei keinem der Inokulations-Verfahren kann eine schnelle Entwicklung hin zu Leitbildgesellschaften erwartet werden, jedoch gehen die Entwicklungen in diese Richtung. Das nachfolgende Weidemanagement hatte bisher vor allem strukturelle Auswirkungen und dient außerdem als Vektor für epi- und endozoochore Ausbreitung.

1 General Introduction

Lacerta agilis

Helichrysum arenarium



Papaver rhoeas and *Vicia villosa* s.l.

Background

Many open ecosystems in the Central European landscape are a consequence of human influence. These ecosystems mostly developed as a result of various kinds of land use. One historically very common type of land use was low-intensity grazing (mostly by sheep; Poschlod & WallisDeVries 2002). Changes in land-use intensity threaten open ecosystems (Ssymank et al. 1998). Abandonment of traditional agricultural practices or of extensive grazing as well as the intensification of arable farming resulted in a dramatic decrease of semi-natural grasslands in the 20th century (Poschlod et al. 2005; Hooftman & Bullock 2012). This process carries on in the 21st century, and especially in the study area (northern Upper Rhine Valley, Germany) urban development, including traffic routes, also leads to loss of habitats.

Relict plant and animal populations of threatened open habitats are often embedded in a matrix of agricultural land and other sites used by humans, and thus experience fragmentation (Saunders et al. 1991). Fragmentation results in a diminishment of area size and increases the spatial isolation of remnant habitats (Saunders et al. 1991; Hooftman & Bullock 2012).

Spatial isolation reduces the connectivity between remnants, as described by Soons et al. (2005) for anemochorous seed dispersal. Zoochorous dispersal by domestic mammals mostly vanished when livestock ceased to be free ranging (Poschlod & Bonn 1998), as the home range of wild mammals is in Central Europe mostly too small to connect distant patches. Described threats deriving from fragmentation are, amongst others, reduced reproduction in small populations (Fischer & Matthies 1998; Kéry et al. 2000; Jacquemyn et al. 2002), partly linked with pollinator limitation (Steffan-Dewenter & Tscharntke 1997), reduced gene flow (Young et al. 1996) and loss of genetic diversity (Honnay & Jacquemyn 2007), with the final risk of extinction of specialist species in small populations (Fischer & Stöcklin 1997; Kéry et al. 2000). Extinctions are found to be more likely for species with high habitat specificity (Fischer & Stöcklin 1997).

In view of this background, target species and their communities require support in terms of enlarging remnant habitats, connecting fragmented patches to one another and to suitable habitats, (re-)creating habitats and facilitating dispersal and (re-)establishment.

Restoration in general

According to the Society for Ecological Restoration (SER 2004), ecological restoration is defined as ´the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed´. Thereby, not only former functions should be re-established but also the characteristic species, communities and structure (´true´ restoration sensu van Diggelen et al. 2001). For planning a restoration project a reference ecosystem should serve as a model, which can subsequently be used to evaluate the restoration (SER 2004). Research on ecological restoration should focus on developing and testing methods for directing and accelerating the development toward desired target communities and ecosystems (Hölzel et al. 2012).

Restoration: Abiotic constraints

An important factor determining the occurrence of different ecosystems is the soil nutrient status and the associated type of nutrient limitation. According to the studied ecosystem, mostly nitrogen (Olff et al. 1993; Mamolos et al. 2005) or phosphorous (Wassen et al. 2005; Hejcman et al. 2007) are discussed as the limiting factor. Changes in nutrient supply and hence in limitation can influence the floristic composition (Güsewell 2004; Chytrý et al. 2009). Nutrient enrichment can facilitate ruderalisation (Süss et al. 2004) and favour fast-growing perennial grasses (Bakker & Berendse 1999). Enhanced depositions of airborne nitrogen are considered to be responsible for grass encroachment (Bobbink & Willems 1987; Bobbink et al. 1998) and decrease in species diversity (Clark & Tilman 2008; Stevens et al. 2010). Especially ecosystems dependent on nutrient-poor soil conditions are sensitive to enhanced nutrient availability.

Therefore, conversion of arable land or pastures into restoration sites is often impeded by high nutrient concentrations in the soils due to the agricultural use. Restoration of communities adapted to nutrient-poor soil conditions, like the inland sand ecosystems studied in this thesis, requires a reduction of the nutrient excess. Grazing and cutting with hay removal can be performed, but this approach takes decades to reduce nutrients to a sufficiently low level (Bakker et al. 2002). Faster impoverishment can be achieved by topsoil removal (Hölzel & Otte 2003; Klimkowska et al. 2010). Initial trials of topsoil inversion and deposition of deep sand were conducted in our study area (Eichberg et al. 2010). Without abiotic improvement succession may tend to produce ruderalised stands (Stroh et al. 2002).

Restoration: Biotic constraints

Plants depend on two main dispersal strategies: dispersal in time (i.e. stored in the soil seed bank) and dispersal in space (i.e. transportation via different vectors). Both strategies are hampered in most restoration approaches. In most cases the seed bank of restoration sites is depleted in seeds of desired species (Stroh et al. 2002; Bossuyt & Hermy 2003; Bossuyt & Honnay 2008), as species characteristic for grasslands tend to have only transient to short-term persistent seed banks (Krolupper & Schwabe 1998; Stöcklin & Fischer 1999). The contribution of seed rain to dispersal of target species is, as already mentioned above, constrained by fragmentation. Restoration of target grassland communities on ex-arable land by spontaneous succession was sufficient in regions with a high density of adjacent donor sites (Ruprecht 2006; Řehounková & Prach 2008; Albert et al. 2014). In the severely fragmented Central European landscape this possibility is generally not given; non-target species dominated the seed rain of restoration sites (Stroh et al. 2002; Eichberg et al. 2010). Dispersal distance via wind is dependent on initial seed release height, different falling velocities of seeds, height and density of the surrounding vegetation and not least by thermal updraft (Tackenberg et al. 2003a; Tackenberg et al. 2003b).

For nature-conservation approaches livestock grazing has been (re-)introduced in various open ecosystems in the last few decades (Kooijman & van der Meulen 1996; Loucougaray et al. 2004; Wagner et al. 2013); thereby, dispersal via zoochory was (re-)introduced simultaneously. Numerous studies revealed the potential of external (epizoochory) or internal (endozoochory) transport of seeds via domestic mammals, e.g. sheep (Fischer et al. 1996; Wessels et al. 2008), cattle (Mouissie et al. 2005a), horses (Cosyns et al. 2005; Cosyns & Hoffmann 2005) or donkeys (Couvreur et al. 2005a). Post-dispersal observation of endozoochorously dispersed seeds revealed that only a small portion of viable seeds effectively emerged under field conditions. Of these, mostly non-target species emerged from horse and cattle dung (Cosyns et al. 2006); in contrast, Eichberg et al. (2007) found (few) threatened species to be most successful in establishing from sheep faeces. Epizoochorous post-dispersal fate was only rarely investigated. Establishment of an epizoochorously dispersed target species, *Jurinea cyanoides*, was studied by Eichberg et al. (2005) for two years, and incorporation into the soil by trampling was found to be a key mechanism for emergence. When Wessels-de Wit & Schwabe (2010) tested the establishment of a set of typical sand species after epizoochorous dispersal during an eight-month period, almost all species emerged and established.

For the (re-)establishment of target communities on restoration sites in most cases human-assisted introduction of species is essential. Thereby the local provenance of propagules is of great importance (see Kiehl et al. 2010). For the re-creation of semi-natural grasslands in Central Europe different methods have been tested; common methods are sowing of site-specific seed mixtures, transfer of seed-containing plant material (fresh or dried hay, raked plant material), brush harvesting, transfer of sods or seed-containing soil (but thereby the donor community gets destroyed) and plug planting (reviewed in Hedberg & Kotowski 2010; Kiehl et al. 2010). Particularly application on bare soil revealed promising results (Hölzel & Otte 2003; Kiehl & Pfadenhauer 2007; Klimkowska et al. 2007; Eichberg et al. 2010). The use of mown or raked plant material has the benefit of transferring on average more species, many of them rare, than sowing (Kiehl et al. 2010); besides, the transfer of bryophytes and lichens is enabled (Jeschke & Kiehl 2006). Additionally, layers of applied plant material can act as erosion control (Kirmer & Mahn 2001) and create safe sites for seedling establishment if the layer is not too thick (Eckstein & Donath 2005). Finally, removal of plant material can have positive effects for the donor site as it creates gaps for plant recruitment, though acting as a management measure as well.

Besides restoration strategies for vascular plants or plant communities, a special approach is the (re-)establishment of biological soil crusts (BSCs). These soil-surface communities, also referred to as cryptogamic, microbiotic or microphytic soil crusts (see Harper & Marble 1988), are assemblages of cyanobacteria, algae, bryophytes, lichens and fungi aggregating with soil particles and stabilizing the uppermost millimetres of the soil (Belnap et al. 2001a). Though the main distribution of BSCs is in arid and semiarid regions throughout the world (see Belnap & Lange 2001), they also occur in temperate regions where edaphically dry conditions or local disturbance enable their presence (Pluis 1994; Lukešová 2001; Langhans et al. 2009a). The (re-)establishment of BSCs can take place by natural colonization via air or may be promoted by human-induced measures. Spontaneous appearance of BSCs is a natural process during early ecosystem development (Schwabe 1974; Yoshitake et al. 2010) and occurs also on (human-)disturbed sites (Spröte et al. 2010; Huang et al. 2011). To speed up the (re-)colonization of BSCs, various rehabilitation methods were tested and applied, which can be divided into three broad categories: artificial soil stabilization techniques, resource augmentation techniques (i.e. modifying soil nutrients or moisture) and inoculation-based techniques (reviewed by Bowker 2007). Inoculation includes the use of cultures (single- or multi-species) or of soil crusts derived from a donor site. Mass culturing of particular isolated cyanobacteria and spraying the solution onto unconsolidated soil was shown to develop

biological crusts at least in the short term (Chen et al. 2006; Malam Issa et al. 2007). Enhanced recovery of BSCs was also achieved using a soil-crust slurry (in combination with watering and fertilization; Maestre et al. 2006) or crushed BSC material (Belnap 1993), though Belnap (1993) suggested full recovery would take decades. Transplanting BSC pieces was discussed as a way to establish founder populations of particular taxa (Scarlett 1994; Bowler 1999).

Management

For the long-term persistence of (restored) semi-natural habitats regular management is crucial (Hölzel et al. 2012). Especially sand ecosystems depend on small-scale disturbance to maintain the open vegetation structure (Jentsch et al. 2002a). Lack of interference may result in succession toward non-targeted vegetation types. Besides mowing, mulching and controlled burning (Moog et al. 2002), which will not be discussed here, extensive grazing by different livestock species is a management tool used for the conservation of semi-natural grasslands (Bakker 1998; Süss et al. 2009; Plassmann et al. 2010).

Grazing has, dependent on the studied system, the ability to slow down the encroachment of dominant tall grasses or even decrease their cover (Kooijman & van der Meulen 1996; Schwabe et al. 2013). Avoiding expansion of shrub species and decline of open ground are other targets tackled by grazing (Lamoot et al. 2005a; Lamoot et al. 2005b), as well as counteracting ruderalisation (Stroh et al. 2002). Furthermore, extensive grazing was shown to enhance species richness (Süss & Schwabe 2007). Trampling, scratching and wallowing of the livestock creates gaps in the vegetation and thereby generates microsites for seedling establishment (Martin & Wilsey 2006; Mitchell et al. 2008). Open gaps, e.g. wallowing places of donkeys, facilitated the establishment of therophytes in sandy grassland (Zehm et al. 2004).

Outline

In the following, aspects of restoration of calcareous sandy grassland with regard to soil nutrients, overcoming dispersal limitation and follow-up management will be examined as well as the effects of transplanted stable biological soil crusts on initial crust development, and the impact of fertilization on vegetation-nutrient status in intact sandy grassland.

For the study on re-establishment of sandy grassland a broad-scale restoration project was started in 2009/2010 in the northern Upper Rhine Valley (**Chapter 2**). An ex-arable field adjacent to the nature monument ´Apfelbachdüne´ was deposited with deep sand for abiotic improvement. The impact of substrate on the restoration success was analysed by deposition of deep sand with different substrate conditions; subsequently, soil analyses were conducted and the soil seed bank was sampled. On the two newly modelled dune sites the suitability of fine-scale inoculation with raked plant material was tested for restoring sandy grassland on the entire restoration area on the basis of yearly relevés. The ability of seed rain for dispersal was analysed during the first two years. Additionally, donkeys were employed for management of the sites and I investigated their impact on vegetation development.

An experiment on sheep epizoochorous dispersal was the basis of **Chapter 3**. I investigated if epizoochorous introduction of a set of species had a lasting impact on establishment and persistence of these habitat-typical species on newly created bare soil patches. Additionally, the persistence of spatial distribution was analysed for the introduced species. The experiment was started in 2005 (see Wessels 2007) and monitored by S. Wessels and I. Retta until 2007. Since then, I continued the experiment and extended the approach by analysing the community development in comparison to the surrounding vegetation and an adjacent nature reserve.

In **Chapter 4**, I was concerned with the establishment of biological soil crusts during primary succession. Again, the restoration area ´Apfelbachdüne´ was used as study site. I transplanted small squares of stable BSCs, obtained from a donor site, onto the nutrient-poor restoration site and examined whether the initial development of BSCs was affected by these transplants depending on distance and time. I compared community composition and taxa numbers of the transplants with samples obtained from three distances to the transplants and control plots at intervals of 0, 12 and 24 months after transplantation. The studied taxa included cyanobacteria, eukaryotic algae, bryophytes and lichens.

A long-term experiment on nutrient addition (started in the year 2000 and conducted in early successional sandy grassland) was the basis for exploring the nutrient status in phytomass in

relation to different added nutrients (**Chapter 5**). I analysed data on phytomass weights and nutrient concentrations for evaluating changes in phytomass production, nutrient concentrations and N:P ratios of different plant functional types as a result of different nutrient treatments. Additionally, I continued the analysis of the successional pathways (see Storm & Süss 2008; Faust et al. 2012) and included three further study years.

In **Chapter 6** a general discussion is presented, connecting the results of the four studies.

Study area

All study sites and reference areas are located in the ´Darmstadt-Dieburger Sandgebiet´ in the surrounding of Darmstadt, Germany (Fig. 1.1). The restoration area ´Apfelbachdüne´ (**Chapters 2 and 4**) was created subsequent to the homonymous nature monument north of Gräfenhausen. Inoculation material for this restoration area was obtained from the donor site ´Standortübungsplatz´, bearing mainly pioneer stages of Koelerion glaucae vegetation. Parts of the reference site ´Seeheimer Korridor´ south of Darmstadt were restored with donor material of the same site in 2005. Biological soil crusts transplanted to the restoration site 1 of the ´Apfelbachdüne´ were gathered from the eastern part of the nature reserve ´Ehemaliger August-Euler Flugplatz von Darmstadt´. The restoration site ´Streitgewann´ (**Chapter 3**) is located between the two nature reserves and Fauna-Flora-Habitat directive areas ´Ehemaliger August-Euler Flugplatz von Darmstadt´ and ´Griesheimer Düne und Eichwäldchen´ and served as a ´stepping stone´ between these areas. ´Griesheimer Düne und Eichwäldchen´ served as reference area for the ´Streitgewann´ site and is characterized by Allio-Stipetum and, in small patches, Koelerion glaucae vegetation. The study sites of the ´Ehemaliger August-Euler Flugplatz von Darmstadt´ (**Chapter 5**) were located in the eastern part of the nature reserve mainly covered by Koelerion glaucae vegetation.

Fig. 1.1: Location of the study areas, reference and donor sites. Image by Google Earth 2013.

2 Restoration of a newly created inland-dune complex as a model in practice: impact of substrate, minimized inoculation and grazing

Koeleria glauca

inoculated and ungrazed plot on restoration site 1 ´Apfelbachdüne´, 2012

grazing donkey

2.1 Abstract

In Central Europe stands of the Koelerion glaucae vegetation complex are threatened and mostly highly fragmented. Knowledge about the impact of abiotic and biotic measures to restore this vegetation complex is crucial. Therefore, an inland sand dune complex (around 2 ha) was created in 2009 as a study model in the Upper Rhine Valley (Germany), which included sites with different substrate conditions as well as grazing impact and minimized inoculation with plant material.

The restoration area is divided into two halves with different substrate conditions (sites 1 and 2), on which inoculation with raked plant material and grazing by donkeys was studied on systematically arranged plots with randomised treatment distribution (32 plots). Additionally the whole area was monitored by a grid-plot approach to show the floristic background (43 plots). Minimized inoculation was conducted with rare Koelerion glaucae plant material in small plots covering around 5 - 7 % of the restoration sites. During the four-year study, vegetation development was recorded and examined in relation to the donor site and an older restoration site. Soil seed bank and seed rain in the newly deposited restoration sites were also investigated, as well as the epi- and endozoochorous seed-dispersal by donkeys. Target species ratios (TSR) were calculated to estimate the restoration success. We used mixed linear models and detrended correspondence analysis for data evaluation.

Substrate conditions had an impact on the number of target species and on phanerogam and cryptogam cover. Inoculation enhanced both number and – without grazing – cover of target species since the first year. On not-inoculated plots and on grid-plots, target-species numbers increased gradually. Grazing by donkeys did not affect target-species numbers, but had a decreasing effect on target-species cover. Grazing reduced bryophyte cover, especially on inoculated plots. DCA revealed development of the experimental plots towards the donor site, as has occurred on the older restoration site. Soil seed bank and seed rain were characterized by ruderal species, and did not show similarities to the donor site. Zoochory revealed some target species to be effectively dispersed by donkeys.

Minimized inoculation is suitable to overcome seed limitation and build up starter populations of target species for the colonization of larger restoration sites. However, within four years species composition of the donor site was not achieved. Grazing by donkeys had mainly structural effects for the studied time period.

2.2 Introduction

Central European semi-natural grasslands on calcareous sandy soils are characterized by threatened plant communities and protected by the Fauna-Flora-Habitat directive of the European Union (Natura 2000-Code 6120, Ssymank et al. 1998). Threats mostly arise from changes in land use such as abandonment or agricultural intensification (Poschlod et al. 2005), leading to degradation (e.g. by grass encroachment) or losses of Koelerio-Corynephoretea and Festuco-Brometea stands with their habitat-typical species. The remaining habitats are highly fragmented and face various threats like reproductive impairment (Aguilar et al. 2006), genetic depletion and even local extinction (Fischer & Stöcklin 1997). Restoration should therefore concentrate on enlarging and connecting the few remaining sandy grassland sites.

A serious problem for restoration in this habitat type is the almost complete absence of sites with appropriate abiotic conditions. Therefore a higher degree of intervention (Walker et al. 2014) is necessary ´to achieve a desired diversity of species´ and communities. Most sites considered for restoration are eutrophicated due to former use as arable fields, constraining the re-establishment of semi-natural grasslands adapted to nutrient-poor soil conditions. Elevated concentrations of both nitrogen and phosphorus in the soil affect restoration adversely. Nitrogen is the limiting factor in our ecosystem type (Storm & Süss 2008); enhanced availability was shown to accelerate succession which differed from the typical pathway for sandy grassland (Faust et al. 2012). The soil phosphate-phosphorus concentration could be related to a decline of the target grass species *Stipa capillata* above a threshold of approximately 20 mg kg^{-1} in our study areas (Süss et al. 2004). Target pioneer species can be outcompeted by ruderal species in restoration sites with high soil phosphate concentrations (Stroh et al. 2007).

Therefore, the abiotic conditions have to be restored at first to obtain suitable soil conditions for establishment. To reduce nutrients like soil phosphate, topsoil removal was successfully applied in various studies (Allison & Ausden 2004; Jaunatre et al. 2014; Olsson & Ödman 2014), but this technique is cost-intensive (Török et al. 2011). An alternative approach – tested in our study area – is the deposition of sand from > 1 m depth, which also creates nutrient-poor soil conditions (Eichberg et al. 2010).

Beyond the low nutrient concentrations also low seed numbers characterize these deep soil layers (Eichberg et al. 2010). As seed banks of formerly arable fields are mostly dominated by weedy species (Hutchings & Booth 1996) and most target species have only low dispersal

distances (Jentsch & Beyschlag 2003), successful spontaneous establishment requires a target community in the immediate vicinity (Donath et al. 2003; Stroh et al. 2007). To overcome seed limitation various measures to introduce species have been tested (Kiehl et al. 2010). Convincing results were obtained, e.g., by spreading raked or mown plant material onto restoration sites (Kiehl & Pfadenhauer 2007; Baasch et al. 2012) but also by transfer of seeds of single plant species (Fritsch et al. 2011). Raked material has the benefit that not only seeds of phanerogams are transferred but also bryophytes and lichens (Eichberg et al. 2010; Jeschke 2012). The transferred plant material is usually applied in stripes (Hölzel & Otte 2003; Donath et al. 2007) or, at smaller restoration sites, on the whole site (Eichberg et al. 2010).

To enhance and maintain restoration success, follow-up management has to be applied to the restoration site (Kiehl et al. 2010). Calcareous sandy grasslands depend on regular disturbance, which is guaranteed by traditional management (Fischer et al. 1996; Langhans et al. 2009a). In our study area management of intact sandy grasslands comprises sheep and/or donkey grazing (Süss & Schwabe 2007). Donkeys create gaps in the vegetation by trampling and wallowing (Süss & Schwabe 2007), which is especially important in consolidated sandy grassland, and reduce competitive graminoids (Lamoot et al. 2005a). Apart from the grazing impact, large herbivores can serve as dispersal vectors via epizoochory (Couvreur et al. 2005a) and endozoochory (Cosyns et al. 2005; Mouissie et al. 2005b; Rosenthal et al. 2012). Little is known about the impact of grazing donkeys on newly created restoration sites.

The aims of the present four-year study were to test the combination of abiotic restoration, minimized biotic restoration and a subsequent grazing by donkeys, to transform a former arable land into calcareous sandy grassland. On the abiotic side, deposition of deep sand on a larger restoration site was so far conducted in one study only (Eichberg et al. 2010), but the impact of substrate conditions was not investigated there. On the biotic side, we tested a minimized application of raked plant material, as donor sites for high quality plant material are small and extensive removal of plant material may negatively affect populations of target species at the donor sites. The use of donkeys for management grazing and their impact as dispersal vectors on newly created restoration sites is almost unknown.

To highlight these aspects for restoration practice the following questions were addressed:

(1) What is the impact of substrate condition on development of sandy grassland?

(2) Is the inoculation of small plots sufficient to restore sandy grassland in a larger area in a period of four years?

(3) Which role does zoochorous dispersal by donkeys play for target species?

(4) How does grazing by donkeys affect the vegetation development?

2.3 Methods

2.3.1 Study area

Located in the northern Upper Rhine Valley, Germany, the study area is characterized by calcareous, nutrient-poor sandy soils (arenosol). The sand originates from aeolian deposits of the Rhine terraces from the late glacial and early postglacial period (Ambos & Kandler 1987). Since the Middle Ages anthropo-zoogenic impact (military training areas, pastures) has preserved the open structure of these habitats (Zehm & Zimmermann 2004). Being in the biogeographic transition zone between subatlantic, subcontinental and submediterranean influence, the co-occurrence of species of these biogeographic zones is remarkable, e.g. *Koeleria glauca* ((sub-)continental), *Corynephorus canescens* (suboceanic) and *Silene conica* (submediterranean). Nowadays, only fragments of the specific vegetation complexes and plant communities Koelerion glaucae Volk 1931 (priority habitat 6120 ´Xeric sand calcareous grasslands´) and Allio-Stipetum capillatae Korneck 1974 (priority habitat 6240 ´Sub-pannonic steppic grasslands´) persist (Süss et al. 2004; Langhans et al. 2009a).

The mean annual temperature is 9.7 °C with a mean annual precipitation of 658 mm (data from Frankfurt/Main airport, 1961-1990; Deutscher Wetterdienst, www.dwd.de).

2.3.2 Restoration sites and abiotic restoration measures

The restoration area (´Apfelbachdüne´; 8°35' E, 49°56' N; Fig. 2.1) is situated about 20 km south of Frankfurt/Main. The restoration methods were employed as a compensation measure for a construction project in this area. Until summer 2009 the area was used as arable field; in autumn 2009 it was abiotically restored by depositing deep sand (layer thickness 1-3 m). Restoration site 1 (S1; 1.1 ha; around 20,000 m^3 sand) received high-quality sand (assignment criterion Z0 according to LAGA-M 20) of low nutrient status; the adjacent restoration site 2 (S2; 0.8 ha; around 14,000 m^3 sand) received sand with partly higher phosphate concentrations. Detailed soil data are given in the ´Results´ section. The deep sand was transferred from two construction sites, whereupon the sand of S2 was temporarily stored above-ground and was thereby contaminated with a small amount of silt.

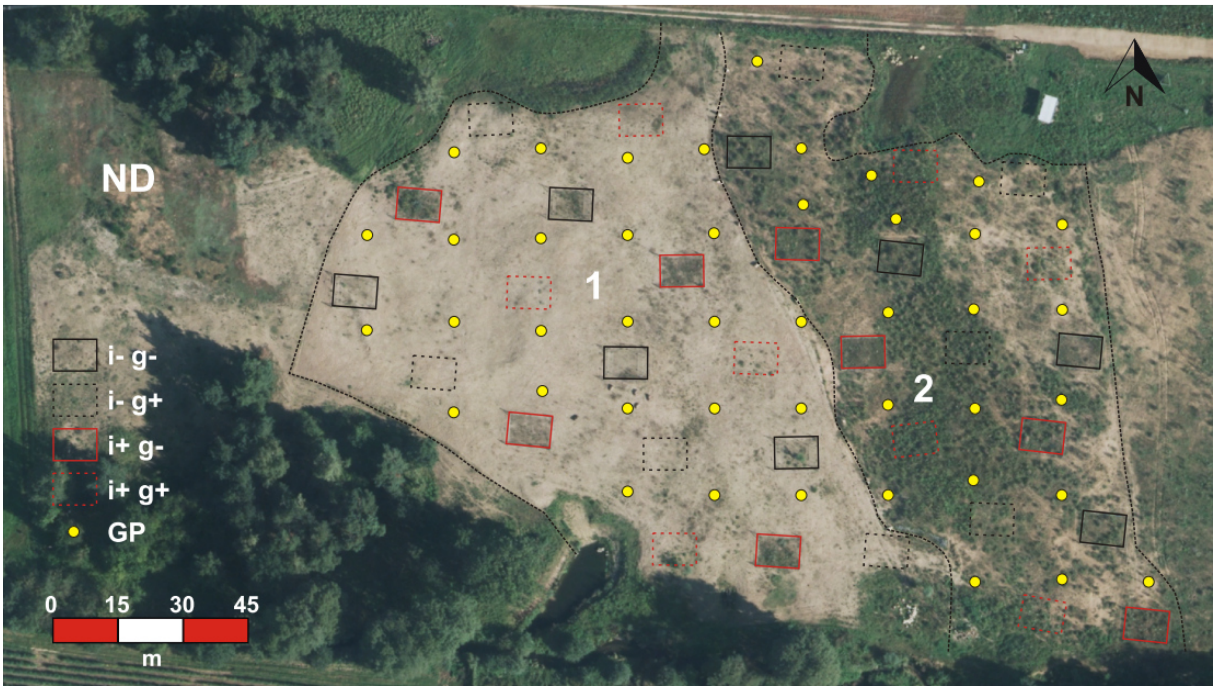


Fig. 2.1: Arrangement of the study plots and grid plots on the restoration sites. ND = old nature monument ´Apfelbachdüne´; 1 = restoration site 1; 2 = restoration site 2. i- g- = not inoculated, not grazed; i- g+ = not inoculated, grazed; i+ g- = inoculated, not grazed; i+ g+ = inoculated, grazed; GP = grid plot. The aerial photograph of the restoration sites (May 2011) was provided by the ´Hessische Verwaltung für Bodenmanagement und Geoinformation´. Creation of the base map was kindly supported by the ´Amt für Bodenmanagement Heppenheim´ (training department).

2.3.3 Experimental design

In total, 32 systematically arranged plots with randomised treatment distribution were installed on the restoration area (Fig. 2.1). On each of the restoration sites 1 and 2, eight plots were inoculated with raked plant material and eight were left untreated, respectively. In each case four inoculated and four untreated plots were fenced against grazing by donkeys. To prevent drift of the material by wind, inoculation material was watered with stream water directly after spreading and pressed to the ground with a roller. The plots were 70 m^2 in extent with a relevé area of 25 m^2 in the centre. Inter-plot distance was at least 15 m on restoration site 1 and 10 m on restoration site 2 (measured from the outer plot edges). Additionally, 43 grid-plots (´GP´; à 25 m^2) were installed (not inoculated, grazed) covering the whole restoration area systematically (site 1: 23 grid-plots; site 2: 20 grid-plots) to show the ´floristic background´ of the whole area.

Since 2010, both restoration sites were grazed separately by a flock of three (autumn 2010) to five (2011-13) donkeys in summer. Both sites were grazed as long as an acceptable food

supply could be assured. The restoration sites were grazed alternately in multiple short-term periods. Total grazing time was from two (2010/13) to ten (2011) weeks on restoration site 1 and from five (2010) to 14 (2011) weeks on restoration site 2.

2.3.4 Donor site

The donor site for the inoculation material (´D´ for Standortübungsplatz; 8°36' E, 49°51' N) is bearing mainly pioneer stages of Koelerion glaucae vegetation and is situated about 13 km south of the restoration sites. In March 2010 the donor site was treated with a swather and subsequently the loosened plant material was raked by hand. The collection area for each of the restoration sites was about 290 m^2 in extent; the raked material was used to inoculate 560 m^2 on each restoration site. This corresponds to an inoculation area of 5 % on S1 and of 7 % on S2. The inoculation density was about 740 ± 44 g m^{-2} (mean ± SE; n = 144) of air-dried inoculation material.

2.3.5 Soil analysis

Soil samples were collected in December 2009. Sampling points were based on the grid plot approach; 51 samples were taken on S1 and 39 samples on S2. Sampling was conducted using an Eijkelkamp liner sampler (diameter 4.7 cm; Giesbeek, NL); sampling depth was 11-16 cm. The samples were kept cool, sieved (2 mm) within 24 h and frozen (-18 °C) until extraction. Phosphate (P) was measured in calcium acetate/calcium lactate extracts (CAL; 10 g soil + 200 ml) according to VDLUFA (1991). P analysis was carried out photometrically (Segmented Flow Analyser SAN+, Skalar analytical, AA Breda, NL). Total nitrogen (N_{total}) was analysed by elemental analysis (Model 1400, Carlo-Erba, Milan, IT). pH values were measured in 0.01 mol l^{-1} calcium chloride after centrifugation.

2.3.6 Soil seed bank

Seed bank samples were taken at the beginning of March 2010 before inoculation of the restoration sites. Per restoration site, 100 samples were taken in a regular grid (based on the grid plots) using an Eijkelkamp liner sampler (see above). Sampling depth was 11-16 cm. By mixing ten individual samples, ten composite samples were obtained per restoration site. Samples were air dried (to eliminate vegetative propagules) and stored at room temperature.

To assess the seed contents a seedling emergence method was used (Eichberg et al. 2006), in which the samples were filled into trays and placed outdoors in the Botanical Garden of the ´Technische Universität Darmstadt´ on a transparently-roofed platform (0.9 m height). The platform was covered by gauze as a protection against aerial seed input and additionally, trays with autoclaved sand were placed between the samples to control for contamination by air-borne seeds. The samples were kept moist and turned every third month. From July 2010 to Nov. 2012, emerging seedlings were identified, counted and removed.

2.3.7 Seed rain

Seed rain was analysed from May 2010 to May 2012 using funnel traps (Kollmann & Götze 1998). Per fenced, non-inoculated plot eight funnel traps were evenly arranged on a 1 m buffer strip around the relevé area. In total, 32 funnel traps were installed per restoration site. Total sampling area per plot was 0.362 m^2. Trap height was 0.9 m above ground level. To avoid direct seed input into the traps, the vegetation surrounding the traps was cut within a radius of ca. 0.5 m as required. Traps were emptied fortnightly. Trapped seeds were identified and counted; determination was conducted by means of a reference seed collection and literature (Beijerinck 1976; Cappers et al. 2006).

2.3.8 Sampling of inoculation material

To test the potential of the inoculation material to transfer species to the restoration sites, samples of the plant material were collected. Per inoculated plot (n = 16) ten plastic boards (à 33 cm x 33 cm) were randomly distributed prior to inoculation. Afterwards, the spread plant material was collected from the plastic boards, dried and stored at room temperature. In total, 80 samples were taken per restoration site. One sample of every inoculation plot was dried at 70 °C for 48 h to obtain the dry weight. The remaining samples were divided into three fractions (phanerogams, cryptogams/litter and inorganic material) to assess the percentage by weight of these fractions on inoculation material. After re-unifying the three fractions to the initial sample, the samples were spread on trays with autoclaved sand to assess the seed contents in a seedling emergence experiment. The trays were placed outdoors in the botanical garden (see section 2.3.6 ´Soil seed bank´). Trays were watered when samples got dry. Further procedure was as described in the ´Soil seed bank´ section.

2.3.9 Vegetation relevés

Since 2010, vegetation relevés were conducted yearly in spring (May; for therophytes) and summer (July/Aug.) on all experimental plots and on the grid plots. The results of these two relevés were combined into one relevé p.a.. For all plant species including bryophytes and lichens both cover-abundance following the extended Braun-Blanquet-scale (BB; according to Barkman et al. 1964) and a percentage scale (0.1, 1, 2, ..., 6, 8, 10, 15, ..., 95, 96, ..., 100 %) were recorded. Additionally, total cover, cover of phanerogams, cryptogams (bryophytes + lichens) and of bare soil were noted.

Nomenclature follows Wisskirchen & Haeupler (1998) for vascular plant species, Koperski et al. (2000) for bryophytes and Scholz (2000) for lichens; syntaxa refer to Oberdorfer (2001).

2.3.10 Comparison of species composition

The vegetation development was set in context with the donor site ´D´ and an older restoration site, which had received inoculation material from the same donor site in 2005 (´RS´ for Seeheimer Korridor; 8°37' E, 49°46' N). Detailed information of the older restoration site is given in Eichberg et al. (2010). For ´D´ vegetation relevés [à 25 m^2; BB] were available for the years 2006-2008; they represent relatively stable pioneer vegetation, which was documented by permanent-plot studies (Süss et al. 2010). For ´RS´ [à 25 m^2; BB] we used the first four years since inoculation (2005-2008) for comparison, as this corresponds to the developmental state of the present restoration site. As management measure the ´RS´ sites were grazed by donkeys.

2.3.11 Zoochory

Epizoochory samples were taken on three dates in 2011 (June and Aug. on site 2, Sept. on site 1). To do this, the fur of the five donkeys was brushed with a fine horse brush at five selected body parts (given are the means of all donkeys): back (0.41 m^2), belly (0.27 m^2), head (0.22 m^2), mane and tail (0.13 m^2), legs (0.47 m^2). Seeds were separated from brushed hair, identified by means of a reference seed collection and literature (Beijerinck 1976; Cappers et al. 2006) and counted. Sampled bryophyte parts were collected, identified and counted as well.

Dung samples were collected in mid June 2012 on restoration site 1. About eight litres of dung were sampled from different dung accumulations and pooled. The samples were washed with tap water on a sieve (10 mm) to remove potentially adhering sand and seeds. The samples were then coarsely crumbled and dried (40 °C) for seven days. Seed contents were quantified using a seedling emergence method. Pots (22 cm x 15 cm) were filled with a layer of sterile potting soil (4 cm) covered by a thin layer of sterile sand (4 mm); on it, a thin layer of dry dung (10 g, ca. 5 mm) was spread. The pots were placed in a greenhouse (20 °C, 20 h light) for eight weeks; samples were watered every second day. Emerging seedlings were identified, counted and removed. When identification was difficult, seedlings were transplanted to separate pots and grown until identification was possible. After the first growing period, a cold stratification was conducted at 4 °C in the dark for six weeks. Subsequently, a second growing period followed with the same conditions as the first.

The field work of the zoochory approaches was done under supervision of A. Schwabe, C. Eichberg and L. Freund by two diploma students (Haußmann 2012; Carrillo 2013).

2.3.12 Data analysis

Vegetation data were analysed with detrended correspondence analysis (DCA) using PC-Ord 6.17 (MjM Software, Gleneden Beach, OR, USA). Included were data of the restoration sites and the sites for vegetation comparison (´D´, ´RS´), as well as data of soil seed bank and seed rain. For the data of the restoration sites, a mean value of the four replicates was calculated for each treatment type. For seed rain data the sum of both years was used. Braun-Blanquet data were transformed to an ordinal scale beforehand (r = 1, + = 2, 1 = 3, 2m = 4, 2a = 5, 2b = 6, 3 = 7, 4 = 8, 5 = 9). Seed bank and seed rain data were transformed to a comparable, weighted scale (1 seed/seedling = 1, 2-9 = 2, 10-49 = 3, 50-99 = 4, 100-499 = 5, 500-999 = 6, 1000-1999 = 7, 2000-2999 = 8, ≥ 3000 = 9). The analysis was run using the options ´downweight rare species´ and ´rescale axes´; the number of segments was 26. To evaluate the percentages of explained variance in the distance matrix, the Relative Euclidean distance was used as recommended by the PC-Ord manual.

Target species ratios (TSR) were calculated as described in Eichberg et al. (2010), as:

TSR_{qual} = number of target plant species/total number of plant species, and

TSR_{quant} = cover sum of target plant species/cover sum of all plant species.

Target species were defined as species with main occurrence in the classes Koelerio-Corynephoretea Klika 1941 and Festuco-Brometea Br.-Bl. et Tx. 1943. Ruderal species were defined as species of the classes Agropyretea intermedio-repentis Müll. et Görs 1969, Artemisietea vulgaris Lohm., Prsg et Tx. in Tx. 1950 and Chenopodietea Br.-Bl. 1951.

To test the effects of ´site´, ´inoculation´, ´grazing´ and ´year´ on various dependent variables, mixed linear models were used (SAS 9.2, PROC MIXED; SAS Institute Inc., CARY, NC, USA; Littell et al. 2006). These models are suitable for analysis of repeated-measures data (Littell et al. 1998), as they allow comparison of the goodness of fit of several covariance structures. The best covariance structure was chosen according to the corrected Akaike criterion (AICC). Degrees of freedom were calculated using the Kenward-Roger approximation (Schaalje et al. 2002).

To test for effects of distance of the grid plots to the next inoculated plot on target species number and target species ratios, Spearman´s rank correlation coefficient was calculated using PROC CORR.

2.4 Results

2.4.1 Soil nutrient status

The soils had nearly identical pH values on both restoration sites; only one value (site 1) was considerably lower than the mean (Table 2.1). N_{total} was very low, although on site 2 on average three-fold higher than on site 1. Phosphate concentrations were very low on site 1. Site 2 had overall higher P concentrations; the values were scattered and covered a relatively wide range.

Table 2.1: Soil data of the restoration sites. Sampling took place in December 2009. Determination was conducted as described in the section 2.3.5 ´soil analysis´ (S1: n = 51, S2: n = 39). min = minimum value; max = maximum value.

	Site 1			Site 2		
	mean ± SE	**min**	**max**	**mean ± SE**	**min**	**max**
PO_4^{3-}-P (mg kg^{-1})	6.87 ± 0.02	1.90	11.33	21.99 ± 2.00	8.14	53.72
pH	7.51 ± 0.05	4.89	7.79	7.55 ± 0.01	7.43	7.67
N_{total} (g kg^{-1})	0.03 ± 0.00	0.01	0.18	0.09 ± 0.01	0.04	0.29

2.4.2 Soil seed bank

The soil seed bank of restoration site 2 had an approximately 5-fold higher seed-density than site 1 and comprised more species (19) compared to S1 (three species; Table 2.2). Most species are ruderal species; target species were only found on S2 with three species and three seedlings. Nearly all species present in the soil seed bank were recorded in the vegetation of the first year of the particular restoration site (Appendix 2.1), except for one species on S1 (*Cardamine hirsuta*; but detected on S2 in 2011) and three species on S2 (two were recorded since 2011 and one since 2013).

Table 2.2: Soil seed bank data, sampled in March 2010 prior to inoculation. The number of seedlings per m^2 is given (mean ± SE; n = 10). Target species are highlighted in red.

Taxa	Site 1	Site 2
Amaranthus retroflexus	12 ± 12	
Cardamine hirsuta	6 ± 6	
Chenopodium album agg.	29 ± 15	63 ± 29
Chenopodium strictum		35 ± 15
Conyza canadensis		17 ± 12
Corispermum leptopterum		6 ± 6
Digitaria sanguinalis		6 ± 6
Eragrostis minor		12 ± 12
Hypericum perforatum		12 ± 8
Lactuca serriola		6 ± 6
Medicago lupulina		6 ± 6
Polygonum aviculare agg.		6 ± 6
Rumex acetosella s.l.		6 ± 6
Saxifraga tridactylites		6 ± 6
Setaria pumila		6 ± 6
Setaria viridis		6 ± 6
Solanum physalifolium		6 ± 6
Sonchus oleraceus		6 ± 6
Taraxacum spec.		6 ± 6
Urtica dioica s.l.		6 ± 6
Veronica arvensis		6 ± 6
indetermined		6 ± 6
Sum	**46 ± 27**	**225 ± 73**

2.4.3 Seed rain

During the two years of seed rain investigation, in total seeds of 58 species were trapped (Table 2.3). Except for seven non-target species, all species were present in the vegetation of at least one of the restoration sites (Appendix 2.1). On restoration site 1 a total of 29 species were recorded in both investigated years, on restoration site 2 species numbers were higher during the two years (in total 43 species). Target species were underrepresented on the two restoration sites, according to both taxa and seed numbers. The seven trapped target species reflected less than 0.1 % of all seeds. Ruderal species dominated the seed rain, accounting for more than 50 % of the trapped species and seeds on the particular restoration site. The most frequently trapped seeds on S1 and S2 were those of the ruderal forb *Conyza canadensis*.

Table 2.3 (next page): Seed rain data of both restoration sites, collected via funnel traps (0.9 m above ground). year 1 = May 2010 to April 2011; year 2 = May 2011 to April 2012. The number of seeds per m^2 is given for each site (mean of the plots ± SE; n=4). Target species are highlighted in red.

Taxa	Site 1		Site 2	
	year 1	year 2	year 1	year 2
Conyza canadensis	1181 ± 158	886 ± 160	1821 ± 1035	3330 ± 327
Betula pendula	278 ± 119	1925 ± 948	54 ± 4	222 ± 99
Salix spec.	171 ± 16	134 ± 9	241 ± 18	169 ± 30
Oenothera biennis agg.	1 ± 1	73 ± 32	1 ± 1	200 ± 43
Alnus glutinosa	21 ± 12	126 ± 90	12 ± 7	95 ± 45
Typha latifolia	46 ± 21	6 ± 5	70 ± 21	15 ± 2
Melilotus albus				133 ± 46
Chenopodium cf. *album*	19 ± 13	1 ± 1	67 ± 35	1 ± 1
Populus spec.	14 ± 5	6 ± 2	5 ± 1	5 ± 2
Phragmites australis		1 ± 1	3 ± 2	23 ± 10
Sisymbrium altissimum			28 ± 27	
Pinus sylvestris	6 ± 3	8 ± 6	2 ± 1	3 ± 1
Oxalis dillenii			2 ± 2	15 ± 12
Solanum nigrum	7 ± 3		7 ± 3	2 ± 1
Cirsium arvense	4 ± 2	1 ± 1	6 ± 3	
Epilobium spec.	1 ± 1	1 ± 1	1 ± 1	6 ± 2
Solidago canadensis		3 ± 2		3 ± 2
Tussilago farfara		1 ± 1	1 ± 1	2 ± 1
Hypochaeris radicata	2 ± 2	1 ± 1	1 ± 1	
Solidago gigantea	1 ± 1	1 ± 1	1 ± 1	
Sonchus asper	2 ± 2	1 ± 1		
Taraxacum officinale s.l.		2 ± 1		1 ± 1
Corispermum leptopterum			2 ± 1	1 ± 1
Atriplex sagittata			3 ± 2	
Viola arvensis			3 ± 1	
Artemisia vulgaris				3 ± 3
Daucus carota				3 ± 2
Bromus tectorum	1 ± 1	1 ± 1		
Holcus lanatus	1 ± 1	1 ± 1		
Lactuca serriola	1 ± 1	1 ± 1		
Epilobium cf. *ciliatum*	1 ± 1			1 ± 1
Senecio spec.		1 ± 1		1 ± 1
Cirsium cf. *vulgare*	2 ± 1			
Vicia hirsuta				2 ± 1
Bryonia dioica	1 ± 1			
Epilobium lamyi	1 ± 1			
Hieracium pilosella	1 ± 1			
Rubus fruticosus agg.	1 ± 1			
Corynephorus canescens		1 ± 1		
Digitaria sanguinalis		1 ± 1		
Rumex acetosella s.l.		1 ± 1		
Senecio vernalis		1 ± 1		
Apera spica-venti			1 ± 1	
Cardamine hirsuta			1 ± 1	
Chenopodium spec.			1 ± 1	
Humulus lupulus			1 ± 1	
Papaver dubium/rhoeas			1 ± 1	
Rumex thyrsiflorus			1 ± 1	
Senecio inaequidens			1 ± 1	
Senecio cf. *vulgaris*			1 ± 1	
Berteroa incana				1 ± 1
Cerastium semidecandrum				1 ± 1
Erophila verna				1 ± 1
Medicago minima				1 ± 1
Poa compressa				1 ± 1
Rumex obtusifolius				1 ± 1
Sonchus cf. *oleraceus*				1 ± 1
Verbascum phlomoides				1 ± 1
Vicia cf. *angustifolia*				1 ± 1
Vicia cracca				1 ± 1
Vicia lathyroides				1 ± 1
indetermined		1 ± 1	1 ± 1	
Sum	**1761 ± 235**	**3184 ± 738**	**2336 ± 1014**	**4241 ± 379**

2.4.4 Inoculation material

The air-dried inoculation material consisted by weight of 86 % inorganic material (mainly sand), 12 % cryptogam phytomass + litter and 2 % plant material of phanerogams. By the seedling emergence method in total 50 phanerogam species were detected in the inoculation material. Thereof 26 species (69 % of the seedlings) belonged to target species (Table 2.4); most frequently germinated *Saxifraga tridactylites*, *Arenaria serpyllifolia* agg. and *Koeleria glauca*. Ruderal species accounted for 34 % of the species (26 % of the seedlings); *Digitaria sanguinalis* was most frequently detected. Seven species (14 %; seedlings: 5 %) were not classified as target or ruderal. According to functional groups, forbs dominated with 37 species (72 %; seedlings: 67 %), followed by graminoids with 12 species (24 %; seedlings: 30 %), one woody plant and one fern (2 %; seedlings: 3 % and 0.2 %, respectively). The fern can most likely be ascribed to a contamination.

Table 2.4 (next page): Plant species detected in the inoculation material. The number of seedlings per m^2 is given (mean ± SE; n = 16). Target species are highlighted in red.

Taxa	No. of seedlings per m^2
Amaranthus retroflexus	0.2 ± 0.1
Arenaria serpyllifolia agg.	18.9 ± 2.0
Artemisia campestris	0.6 ± 0.2
Asplenium ruta-muraria	0.3 ± 0.1
Betula pendula	4.2 ± 1.5
Bromus tectorum	0.1 ± 0.1
Carex hirta	0.1 ± 0.1
Centaurea stoebe s.l.	0.8 ± 0.2
Cerastium semidecandrum	5.4 ± 0.7
Chenopodium album agg.	0.6 ± 0.2
Conyza canadensis	3.5 ± 0.6
Corynephorus canescens	0.1 ± 0.1
Digitaria sanguinalis	0.1 ± 0.1
Diplotaxis tenuifolia	0.1 ± 0.1
Echium vulgare	0.3 ± 0.1
Eragrostis minor	0.3 ± 0.1
Erigeron annuus	0.1 ± 0.1
Erophila verna	0.3 ± 0.1
Geranium robertianum	0.2 ± 0.1
Helichrysum arenarium	0.3 ± 0.1
Holosteum umbellatum	0.3 ± 0.1
Hypericum perforatum	0.1 ± 0.1
Koeleria glauca	9.1 ± 1.4
Koeleria macrantha	0.5 ± 0.2
Medicago minima	0.3 ± 0.1
Oenothera biennis agg.	0.6 ± 0.2
Ononis repens s.l.	0.2 ± 0.1
Petrorhagia prolifera	0.4 ± 0.2
Phleum arenarium	6.4 ± 1.4
Phleum phleoides	0.2 ± 0.1
Poa compressa	0.1 ± 0.1
Potentilla argentea agg.	0.2 ± 0.2
Rumex acetosella s.l.	0.1 ± 0.1
Salix spec.	1.0 ± 0.3
Salsola kali subsp. *tragus*	0.6 ± 0.2
Saxifraga tridactylites	70.1 ± 6.3
Sedum acre	0.2 ± 0.1
Setaria viridis	35.0 ± 5.3
Silene conica	6.0 ± 1.1
Silene otites	0.1 ± 0.1
Sonchus asper	0.2 ± 0.1
Sonchus cf. *oleraceus*	0.3 ± 0.2
Stellaria media	0.1 ± 0.1
Taraxacum officinale s.l.	0.4 ± 0.2
Urtica dioica s.l.	0.2 ± 0.1
Verbascum phlomoides	3.1 ± 0.8
Veronica arvensis	2.9 ± 0.5
Veronica praecox	0.4 ± 0.2
Veronica verna	0.2 ± 0.1
Vicia lathyroides	0.1 ± 0.1
Vulpia myuros	0.1 ± 0.1
indetermined (forb)	2.1 ± 0.3
indetermined (graminoid)	0.9 ± 0.4
Sum	**178.6 ± 12.9**

2.4.5 Vegetation development

Occurrence of target species

Inoculation had a significant positive effect on the occurrence of target species (Fig. 2.2; Table 2.5; Appendix 2.1). From the first to the second study year the number of target species significantly increased in inoculated plots on both restoration sites; thereafter it remained unchanged. In the first year a total of 16 to 18 (ungrazed/grazed; total: 18) target species were recorded on S1 and 24 to 26 (ungrazed/grazed; total: 27) on S2; the numbers increased to 27 and 25 (ungrazed/grazed; total: 29) on S1 and 32 target species (both ungrazed and grazed; total: 35) on S2 in the last year. Grazing had no significant effect on target species numbers. On S1 the number of target species was lower than on S2 throughout the studied period.

On plots without inoculation, the number of target species increased steadily over the four-year study period (Fig. 2.2). Already in the first year two target species were recorded on S1 (both on ungrazed and grazed plots), 13 and nine (ungrazed/grazed; total 15) target species were found on S2. In the last studied year, the numbers rose to ten and seven on S1 (ungrazed/grazed; total: 12) and to 20 and 21 on S2 (ungrazed/grazed; total: 24). Most frequently detected target species across all not-inoculated plots and all years were on S1 *Erodium cicutarium* and *Tortula ruraliformis*, and on S2 *Medicago lupulina* and *Trifolium arvense*. On the grid plots almost completely the same target species were detected as on the

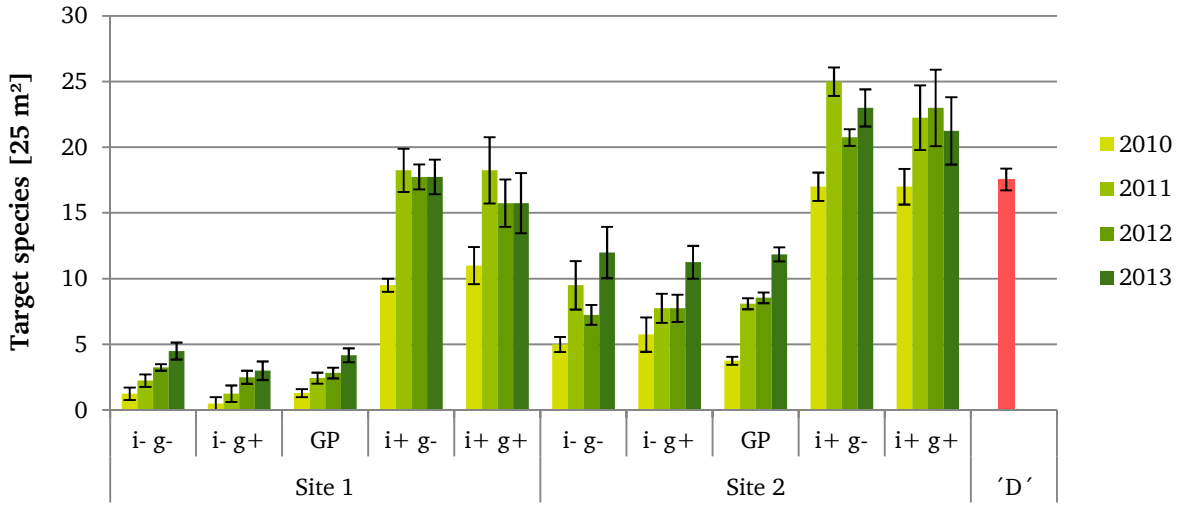


Fig. 2.2: Target species numbers (mean ± SE; n = 4) on the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

study plots. One species, *Myosotis stricta*, was exclusively found on grid plots (S2). The number of target species recorded on all grid plots increased from 2010 (S1: 8, S2: 12) to 2013 (S1: 21, S2: 29). Merging the target species numbers of not-inoculated plots and grid-plots in all years, a total of 37 and 42 target species were detected on S1 and S2, respectively (Appendix 2.1). Of these, two species were exclusively found on S1 and one species on S2. On each of the two restoration sites 11 target species could not be detected outside the inoculated plots.

Target species ratios

The qualitative target species ratio, indicating the proportion of target species to the total species number, was significantly enhanced by inoculation on both restoration sites, although more so on S1 than on S2 (Fig. 2.3; Table 2.5). The increase in TSR_{qual} on inoculated plots from 2010 to the next years was significant and came from an increase of target species on S1 and from a combination of increased target species and a decrease in total species numbers on S2. Grazing on inoculated plots resulted in a significantly lower TSR_{qual} on S2 than on S1. The TSR_{qual} of the not-inoculated plots was higher on S2 than on S1, due to a greater proportion of target species on S2. Reasons for the increase in TSR_{qual} of the not-inoculated plots are the same as on inoculated plots. The TSR_{qual} of the grid plots is in the range of the values found on not-inoculated plots of the particular restoration site; on S1 the grid plots have slightly higher values than the not-inoculated, grazed plots.

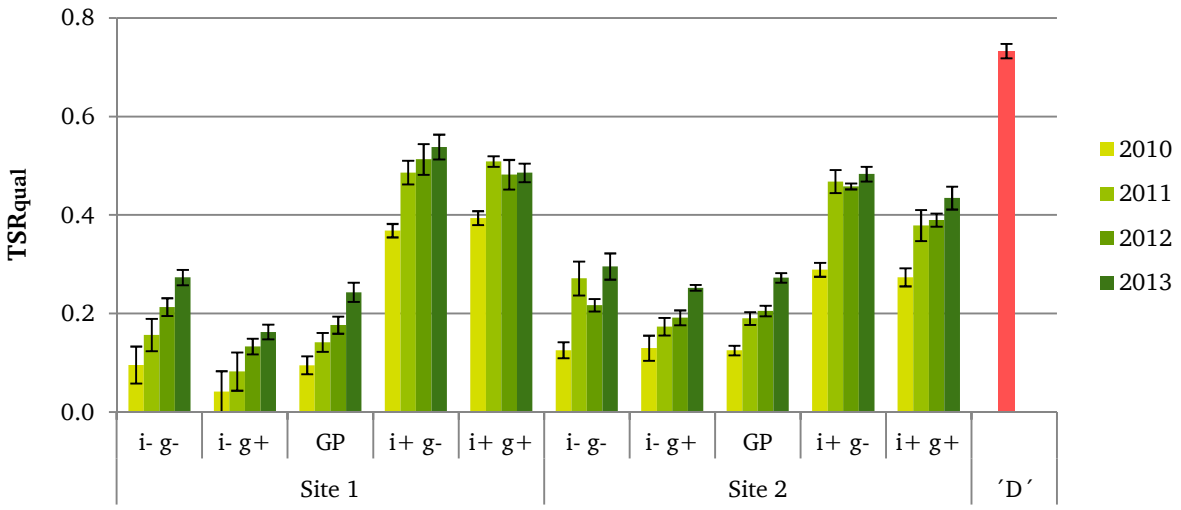


Fig. 2.3: Qualitative target species ratio (mean ± SE; n = 4) of the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

The inoculation-induced increase of the TSR_{quant}, taking plant cover into account, was more pronounced than of the TSR_{qual} (Fig. 2.4; Table 2.5). Inoculation enhanced the TSR_{quant} on S1 significantly more than on S2; on S2 the cover of non-target species was higher overall, which decreased the value. Grazing significantly reduced the TSR_{quant} on inoculated plots dependent on year. To a major extent, this decline was related to a reduction of cover of the bryophyte target species *T. ruraliformis*. Not-inoculated plots had very low TSR_{quant} on both restoration sites; grazing on S2 increased the ratio during the four study years. On grid plots, the TSR_{quant} corresponds to those of the not-inoculated plots of the particular restoration site.

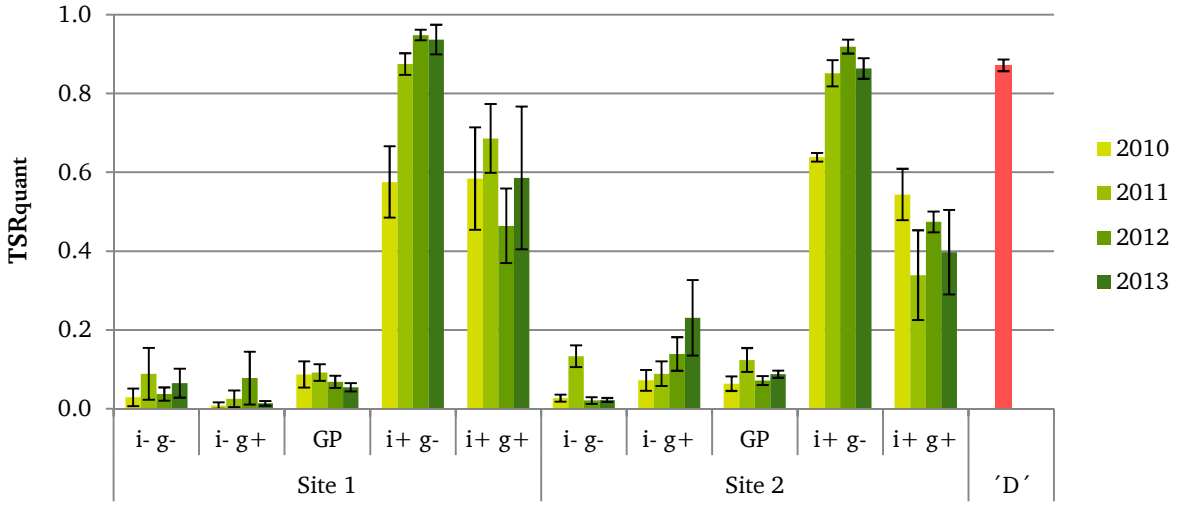


Fig. 2.4: Quantitative target species ratio (mean ± SE; n = 4) on the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

Cover of functional groups

The proportion of open ground remained high on grazed plots on S1, but significantly decreased on S2 despite grazing during the study time (Fig. 2.5; Table 2.5). Grazing on inoculated plots had a significant effect on cover of open ground dependent on year. On S1 grazed plots had similar cover of open ground since the second year, whether they had been inoculated or not. On S2 the cover on inoculated plots was reduced by grazing only in the second year. On not-grazed plots the proportion of open ground declined from the first to the last study year. Grid plots had a proportion of open ground comparable, although slightly higher, to not-inoculated and grazed plots.

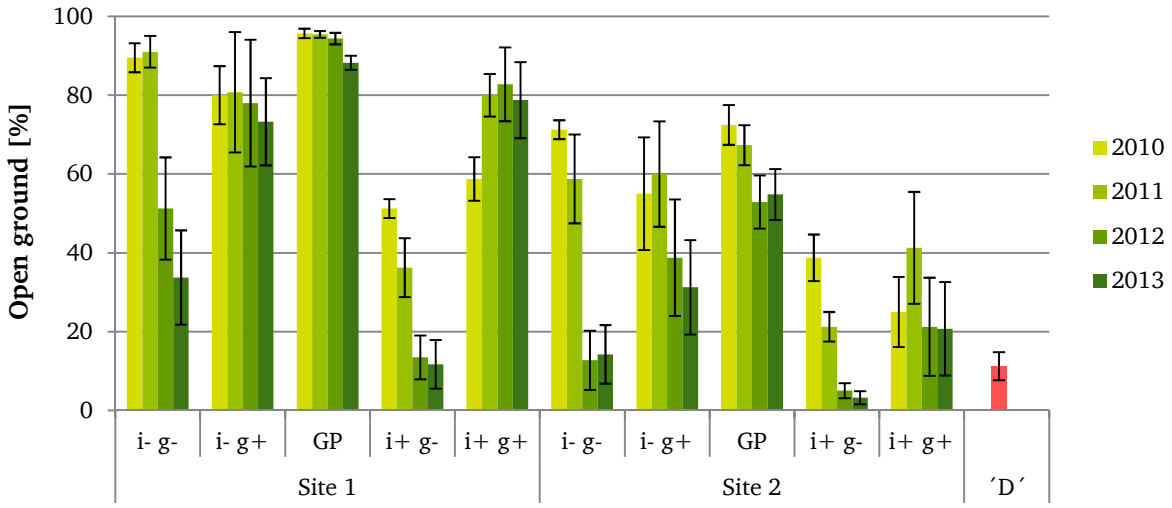


Fig. 2.5: Cover of open ground (mean ± SE; n = 4) on the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

The cover of phanerogams on the two restoration sites was only significantly different according to site and year (Table 2.5). In total, phanerogam cover was higher on S2 than on S1 (Fig. 2.6). On S1 cover tended to increase during the study (except for inoculated, grazed plots); on S2 the cover fluctuated between the years. Grazing on inoculated plots of S1 reduced cover of phanerogams, whereas the cover on S2 was highest on these plots. On S1 the grid plots had the lowest cover of phanerogams, on S2 cover on grid plots was in the range of not-inoculated plots.

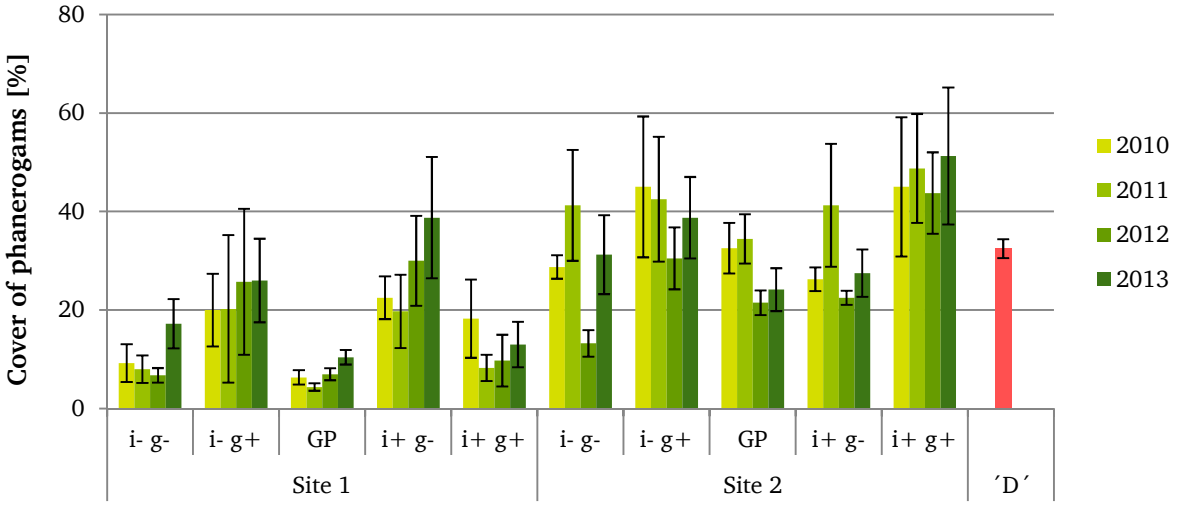


Fig. 2.6: Cover of phanerogams (mean ± SE; n = 4) on the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

Inoculation had already enhanced cover of cryptogams in the first study year (Fig. 2.7; Table 2.5). Without grazing, cover significantly increased during the study period, achieving 73 % on S1 and 90 % on S2 in 2013. Grazing had a significantly decreasing effect on cryptogam cover of inoculated plots, but the decrease was dependent on year. Since 2012, the cryptogam cover of not-inoculated plots increased on both restoration sites, the increase being significantly stronger on S2. Again, the cover was negatively affected by grazing; the impact was greater on S1 than on S2. According to the grid plots the cover of cryptogams corresponds to the cover on not-inoculated, grazed plots of the particular restoration site.

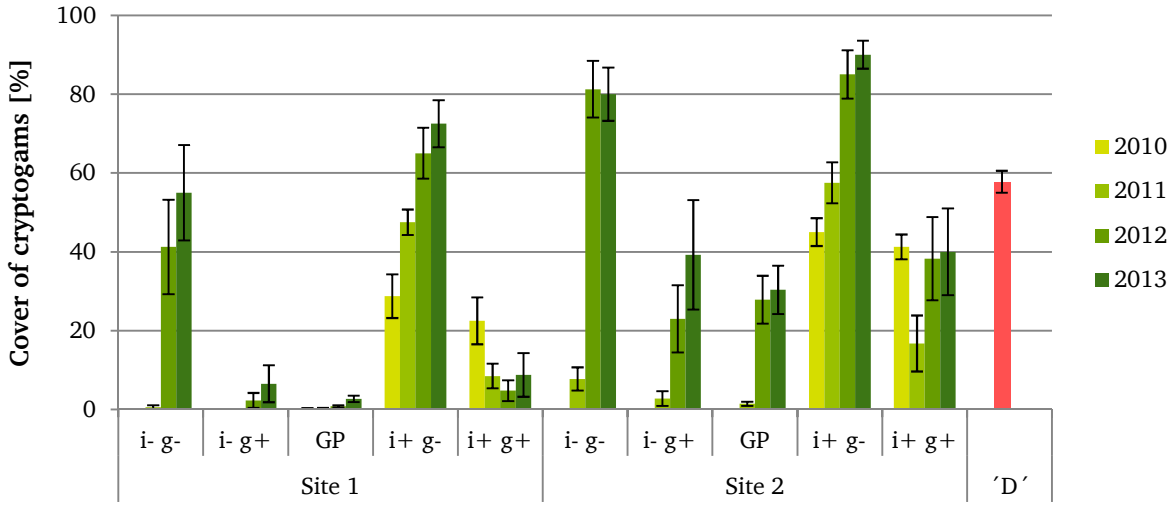


Fig. 2.7: Cover of cryptogams (mean ± SE; n = 4) on the two restoration sites under different treatments (2010-2013) and of the donor site ´D´ (mean of 2006-08). For abbreviations see Fig. 2.1.

Table 2.5: Effects of site, inoculation with plant material, grazing and year on various dependent variables as tested by linear mixed models. TSR_{qual} = qualitative target species ratio; TSR_{quant} = quantitative target species ratio (see section 2.3.12 ´Data analysis´). The values of the degrees of freedom numerator (df num) and degrees of freedom denominator (df de) are separated by the effect of ´year´; target species number (df num: 1, 3; df de: 25.7, 72); TSR_{qual} (df num: 1, 3; df de: 28.9, 31.1); TSR_{quant} (df num: 1, 3; df de: 30.4, 43.6); open ground (df num: 1, 3; df de: 24, 22); phanerogam cover (df num: 1, 3; df de: 24, 72) and cryptogam cover (df num: 1, 3; df de: 24, 22).

	target species no.		**TSR_{qual}**		**TSR_{quant}**		**open ground**		**phanerogam cover**		**cryptogam cover**	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
site	37.41	**<.0001**	0.4	0.5321	0.27	0.6068	22.21	**<.0001**	10.67	**0.0033**	31.58	**<.0001**
inoc	188.04	**<.0001**	647.2	**<.0001**	429.19	**<.0001**	10.86	**0.0031**	0.51	0.4827	43.2	**<.0001**
site*inoc	0.03	0.8707	45.71	**<.0001**	4.83	**0.0358**	0	0.9923	0.01	0.9275	0.29	0.5976
grazed	0.43	0.5166	20.67	**<.0001**	24.63	**<.0001**	9.09	**0.006**	1.39	0.25	98.85	**<.0001**
site*grazed	0.04	0.8451	0.03	0.8603	0.03	0.8642	2.75	0.1104	2.05	0.1651	0.05	0.8169
inoc*grazed	0	0.974	1.9	0.1784	35.59	**<.0001**	2.33	0.1397	0.92	0.3465	5.51	**0.0274**
site*inoc*grazed	0.03	0.8707	4.44	**0.0438**	3.98	0.0551	1.66	0.2099	2.65	0.1167	0.76	0.3916
year	45.32	**<.0001**	91.78	**<.0001**	3.55	**0.022**	46.68	**<.0001**	3.47	**0.0204**	37.75	**<.0001**
site*year	1.14	0.3393	2.41	0.0858	1.4	0.2543	2.5	0.086	5.94	**0.0011**	7.44	**0.0013**
inoc*year	11.33	**<.0001**	6.04	**0.0023**	0.82	0.4916	7.6	**0.0012**	0.56	0.6422	5.29	**0.0067**
site*inoc*year	2.29	0.086	0.69	0.5631	3.29	**0.0293**	0.4	0.7539	0.75	0.5236	1.27	0.3096
grazed*year	1.85	0.1455	4.13	**0.0142**	5.09	**0.0041**	27.36	**<.0001**	1.29	0.2853	19.36	**<.0001**
site*grazed*year	1.76	0.1633	2.46	0.0809	1.98	0.1313	3.38	**0.0364**	1.51	0.2199	1.79	0.1793
inoc*grazed*year	0.2	0.8964	0.48	0.7012	7.4	**0.0004**	3.06	**0.0493**	0.49	0.6909	13.38	**<.0001**
site*inoc*grazed*year	0.88	0.4553	0.12	0.9494	1.66	0.1895	1.7	0.1971	1.07	0.3663	2.93	0.0563

Spreading patterns

The total number of target species was higher on grid plots of S2 than of S1 in 2013 (Fig. 2.8 a), but on both restoration sites no significant effect was found for the distance from the grid plots to the next inoculated plot in any year. Distance had also no significant effect on the qualitative TSR regarding restoration site or year; the TSR_{qual} was very similar on both restoration sites in 2013 (Fig. 2.8 b). Only with regard to the quantitative TSR (Fig. 2.8 c) did distance from the grid plots to the next inoculated plot lead to a significant decrease of the TSR_{quant} on S1 in 2012 (r_s = -0.431, p = 0.040) and 2013 (r_s = -0.519, p = 0.011), but not in the previous years and not on S2.

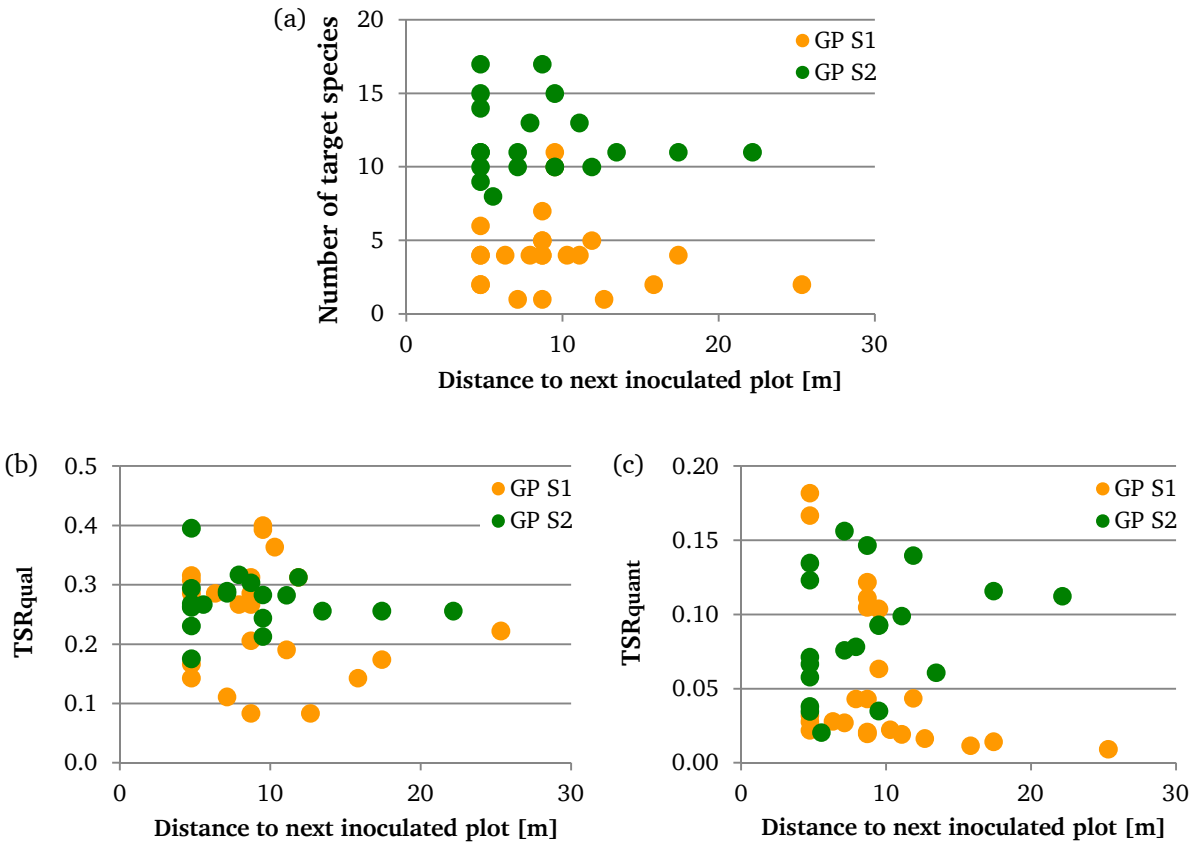


Fig. 2.8: Relations of (a) target species number; (b) TSR_{qual}; and (c) TSR_{quant} of the grid plots to distance to the next inoculated plot for the year 2013. GP S1 = grid plots restoration site 1; GP S2 = grid plots restoration site 2.

2.4.6 Comparison of species composition

Nearly all species (35 species, 90 %) recorded on the donor site (vegetation relevés of 2006-08) were found in the inoculated plots (Appendix 2.1); only four species were not detected in the inoculated plots (two target species: *Alyssum alyssoides* and *Myosotis stricta*). An additional 20 target species were exclusively found on the recipient plots, whereof two were only found on S1 and ten on S2. Three of these 20 species were on the Red List (S1: one species; S2: two species; Korneck et al. 1996).

Comparing the target species ratios of the restoration sites and the donor site revealed differences in qualitative and quantitative TSRs. The mean TSR_{qual} of inoculated plots, with values of 0.54 to 0.49 (ungrazed/grazed) on S1 and 0.48 to 0.43 (ungrazed/grazed) on S2 (in 2013), was still lower than that on the donor site (0.73; mean of 2006-08; Fig. 2.3). In contrast, the TSR_{quant} of inoculated and ungrazed plots had values equal to (S2: 0.86) or even higher than (S1: 0.94) those of the donor site (0.87; mean of 2006-08; Fig. 2.4).

Detrended correspondence analysis revealed a clear separation of the donor site, the restoration sites (S1, S2 and RS) and seed bank and seed rain along the first axis (Fig. 2.9).

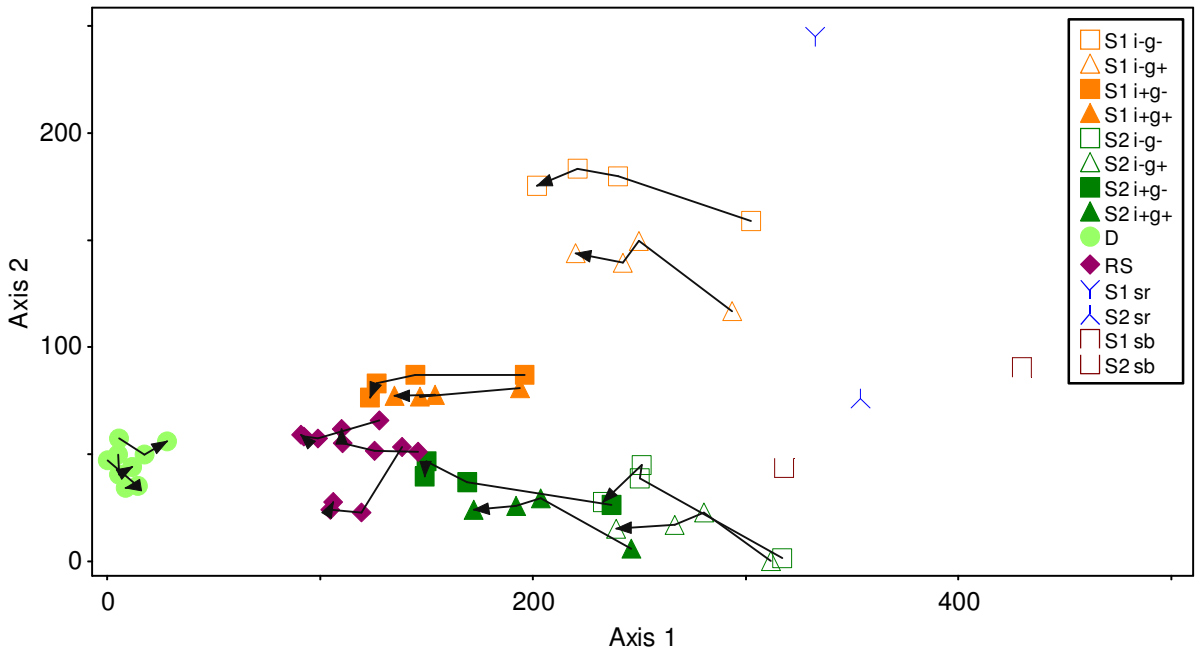


Fig. 2.9: Detrended correspondence analysis (DCA) of the restoration sites S1 and S2 under different treatments (2010-13), soil seed bank and seed rain data of the restoration sites, the donor site ´D´ (2006-08) and another restoration site (´RS´; 2005-08). Time points of experimental plots, donor site and ´RS´ are connected by trajectories. Eigenvalues and in parentheses the percentage of explained variance: axis 1: 0.45 (54.7 %), axis 2: 0.15 (5.1 %), axis 3: 0.12 (10.7 %). For abbreviations see Fig. 2.1; sr = seed rain, sb = seed bank.

The plots of the donor site are closely grouped together on the left side, seed bank and seed rain of the restoration sites are arranged at the opposite side. All restoration sites are located in between, the inoculated plots and the older restoration site being grouped closer to the donor site than not-inoculated plots. The trajectories of both inoculated (incl. RS) and not-inoculated plots tend towards the donor site. The changes in community structure are most pronounced from 2010 to 2011. The second axis separates the two restoration sites; especially according to inoculation and seed rain. The not-inoculated plots of S1 are clearly separated from the inoculated plots and those of S2.

2.4.7 Zoochory

In the epizoochory samples in total seeds of 19 species, one bryophyte species and the pooled group of pleurocarpous bryophytes were detected (Table 2.6). Thereby, the three sampling dates differed in collected species and seed numbers. The number of species declined from June to Sept. (13 to six species); in Aug. most seeds were caught. According to target species, in June all five species were detected (39 % of the species and 73 % of the seeds); most frequently sampled was *Medicago minima*. In Aug. and Sept. the ratio of target species declined to 22 % to 17 %, respectively (both 4 % of the seeds). The Aug. sampling revealed mostly ruderal species (78 %; seedlings: 96 %), especially *Melilotus albus* and *C. canadensis*; in Sept. 50 % of the species and 82 % of the seedlings were ruderals. According to the sampled body parts, most species and most seeds were found on the back of the donkeys (except June: most seeds on the belly).

A total of 88 seedlings of ten species emerged from the endozoochory samples (8 litres; dry weight 1740 g). 60 % of these were target species, accounting for 54 % of the seedlings. The recorded target species were (ordered by frequency) *A. serpyllifolia* agg., *Rumex acetosella* s.l., *Potentilla argentea* agg., *Cerastium semidecandrum*, *Silene conica* and *Phleum arenarium*. Ruderal species accounted for 30 % of the species and 44 % of the seedlings; *Bromus tectorum* most frequently emerged, followed by *Poa angustifolia*. Forbs accounted for 56 % of all seedlings, grasses had a proportion of 44 %. All species detected in the samples (except for *Plantago major*) were present in the vegetation of restoration site 1.

Table 2.6: Epizoochory data for the three sampling dates. Given is the number of seeds per m^2 for the five selected body parts (mean of the donkeys ± SE; n = 5). Target species are highlighted in red. Data provided by A. Haußmann.

	June (Site 2)					August (Site 2)					September (Site 1)				
	back	belly	head	legs	mane & tail	back	belly	head	legs	mane & tail	back	belly	head	legs	mane & tail
Arenaria serpyllifolia agg.	1 ± 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Berteroa incana	-	-	-	-	-	0.5 ± 0.5	-	-	-	-	-	-	-	-	-
Betula pendula	-	-	-	-	-	-	-	-	-	-	6 ± 4	1 ± 1	-	-	-
Chenopodium album agg.	1 ± 1	-	-	3 ± 2	-	-	-	-	-	-	-	-	-	-	-
Conyza canadensis	0.5 ± 0.5	-	-	-	-	21 ± 9	1 ± 1	1 ± 1	3 ± 0.4	9 ± 4	13 ± 2	5 ± 2	2 ± 1	0.4 ± 0.4	3 ± 3
Echinochloa crus-galli	4 ± 3	4 ± 4	-	3 ± 2	-	0.5 ± 0.5	-	-	-	-	-	-	-	-	-
Epilobium cf. *tetragonium*	-	-	-	-	-	0.5 ± 0.5	-	-	-	-	1 ± 1	-	4 ± 3	-	2 ± 2
Matricaria chamomilla	-	3 ± 3	-	-	-	-	-	-	-	-	-	-	-	-	-
Matricaria discoidea	-	-	-	1 ± 1	-	-	-	-	-	-	-	-	-	-	-
Medicago lupulina	0.5 ± 0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medicago minima	2 ± 2	16 ± 11	4 ± 4	14 ± 7	18 ± 12	-	4 ± 4	-	-	-	-	-	-	-	-
Melilotus albus	-	-	-	-	-	64 ± 19	-	1 ± 1	0.4 ± 0.4	9 ± 4	-	-	-	-	-
Oenothera biennis agg.	-	-	-	-	-	3 ± 1	-	10 ± 4	-	-	6 ± 3	-	10 ± 5	-	14 ± 12
Persicaria maculosa	0.5 ± 0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pleurocarpous bryophytes	0.5 ± 0.5	2 ± 1	-	-	-	-	-	-	-	-	0.5 ± 0.5	-	-	-	-
cf. *Poa annua*	2 ± 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rumex obtusifolius	-	-	-	0.4 ± 0.4	-	-	-	-	-	-	-	-	-	-	-
Silene conica	-	-	-	-	2 ± 2	-	-	-	-	-	-	-	-	-	-
cf. *Sisymbrium altissimum*	-	-	-	-	-	-	1 ± 1	3 ± 1	-	2 ± 2	-	-	-	-	-
Tortula ruraliformis	0.5 ± 0.5	1 ± 1	-	1 ± 1	-	0.5 ± 0.5	1 ± 1	-	-	-	0.5 ± 0.5	1 ± 1	-	-	2 ± 2
Typha latifolia	-	-	-	-	-	-	-	-	-	-	4 ± 4	-	-	-	-

2.5 Discussion

2.5.1 Impact of substrate conditions

Substrate condition could be shown to have impacts on phanerogam and cryptogam cover, cover of open ground and on the number of detected target species. Thereby, open ground was reduced and the other parameters were enhanced on restoration site 2 compared to S1. These differences in development of the two restoration sites may be caused by the differences in nutrient status or in the seed bank of the soil substrates.

However, the investigated soil parameters were, except for phosphate on S2, in the range of or even below values measured in target communities of our study area and hence suitable for the re-establishment of calcareous sandy grassland. The pH in both deposited substrates was within the range of values measured in stands of Jurineo-Koelerietum and Allio-Stipetum (Süss et al. 2004). Total nitrogen differed on the two restoration sites with slightly higher values on S2, but overall values were below the 0.28 ± 0.12 g kg^{-1} (mean ± SD; 10-30 cm) measured in sandy grasslands in this region (Süss et al. 2004). The same applies to mineral N values quantified by Lingen (2013) on the restoration sites (0.7 to 0.8 kg N ha^{-1} on S1 and S2, respectively), which were below 3.8 kg N ha^{-1} (0-10 cm) reported by Storm et al. (1998) in stands of Allio-Stipetum. Only the concentration of phosphate-P, which is typically below 15-20 mg kg^{-1} in our study area (Storm et al. 1998), exceeded this threshold on average slightly on S2, but some samples achieved values up to 50 mg P kg^{-1}. Above 40-50 mg kg^{-1}, P is considered to be no more a limiting nutrient in agriculture (Scheffer & Schachtschabel 2002).

The effects of higher P concentrations on vegetation can be, e.g., increased biomass, loss of diversity, especially of endangered species and a facilitation of competitive graminoids (Carroll et al. 2003; Süss et al. 2004; Wassen et al. 2005; Hejcman et al. 2010a). As yet none of these effects can be definitely ascertained for S2 for the investigated time period. The P concentration may have had an influence on the higher vegetation cover on S2 in the first years, e.g. by facilitating legumes (Bobbink 1991; Janssens et al. 1998) like *Melilotus albus* which achieved high cover values. Competitive graminoids were detected on both restoration sites; the frequency and cover increased slightly on S2 during the last study years (data not presented, but see Appendix 2.1).

The factor with a probably stronger differentiating effect for the two substrates in the initial stages is the seed bank. The soil seed bank of S1 was, as expected (Eichberg et al. 2010), extremely poor in species and quantity of seeds. Deep sand of below 1 m depth should be

nearly free of seeds as the number of viable seeds declines with soil depth (Godefroid et al. 2006). Site 2 had an around five-fold higher seed density and more species than S1; at the same sampling depth in Koelerion glaucae stands there were comparable seed densities and even fewer species (Eichberg et al. 2006). The temporary above-ground storage almost certainly caused a contamination of the substrate with seeds, since nearly all species detected in the seed bank were recorded in the first-year vegetation of S2. Even though a strikingly high number of target species was detected on this site – even without inoculation – the high proportion of ruderal species is counterproductive for restoration efforts. Admittedly, the first years of many restoration projects are characterized by weedy annuals (e.g. Jongepierová et al. 2007) but a high proportion of non-target species remained on S2 even in the fourth year.

2.5.2 Minimized inoculation

Application of plant material resulted in a punctual increase of target species number and qualitative and quantitative target species ratios on the inoculated plots since the first studied year. In comparison, on the whole restoration area (i.e. on not-inoculated plots and on grid-plots) these parameters, despite their increasing values, remained much lower, showing that the large-scale restoration of sandy grassland cannot be achieved with this approach in a four-year period but certainly takes more time.

That the application of plant material following abiotic restoration enhances the occurrence of target species was already reported in many other restoration projects (Hölzel & Otte 2003; Kiehl et al. 2006; Donath et al. 2007; Edwards et al. 2007). Quantity and cover of target species on inoculated plots corresponded to the values of the donor site (cf. Eichberg et al. 2010). Inoculated plots developed in the direction of the donor site, but differences remained after four years. Not-inoculated plots developed in the same direction, though the distance to the donor site was greater than in the case of inoculated plots. Approximation of these plots to the donor site may be related to an immigration of target species from inoculated plots to not-inoculated plots (and the surrounding area). During the four study years on both restoration sites 70 % (S1) to 73 % (S2) of all recorded target species were at least once detected outside of inoculated plots. The target species which were not recorded outside of inoculated plots were mostly species found only with few individuals. Burmeier et al. (2011) described as well rather slow colonization velocities in a flood-meadow restoration project, although almost 95 % of species had spread from plant material strips 7-8 years after restoration.

In contrast to Burmeier et al. (2011) the distance between inoculated plot and ´receiver´-plot was not found to be a determining factor for the number of target species, which can have different explanations. On the one hand, the soil seed bank can be – even though only few target species were recorded on S2 – a source for target species irrespective of inoculation. Also, plant material that fell down during the inoculation can be considered as source for target species. These two sources can explain the occurrence of target species which were detected outside of inoculated plots already in the first year. On the other hand, the analysed dispersal vectors can contribute to an unpredictability of dispersal away from inoculation plots. Epizoochorous dispersal distances in the range of tens of meters to a few kilometres were estimated for horses (Couvreur et al. 2005b). Via endozoochory seeds can be transported over several kilometres by large herbivores (Pakeman et al. 2002). Seed rain is also an undirected vector, but it was, in accordance with other restoration projects (Stroh et al. 2002; Freund et al. 2014), dominated by non-target species. Nevertheless, the inoculated plots may serve as a propagule source for target species even though seeds (or dispersal units of bryophytes) were not detected in the seed rain. As the dispersal distances of many target species are only low (Jentsch & Beyschlag 2003), propagules might not have reached the seed traps.

Another factor relevant for evaluating the restoration success is, apart from the quantity of target species, their cover. During the four investigated years the increase in quantity of target species on not-inoculated plots had nearly no effect on enhancing their proportion in total cover. However, this may be linked to the observation in the grid-plot approach that the quantitative TSR declined with distance to the next inoculated plot (at least on S1 in the last two years). In the near vicinity of inoculated plots it might be more likely that target species reach higher cover values, because of the above mentioned low dispersal distance of many target species. This might especially apply to many therophytic and/or early successional target species, e.g. *S. conica*, as they have only low growth-heights or rather small canopies. High cover values can be reached by these species only when occurring in high abundance. Tall-growing target species forming greater canopies emerged almost exclusively on inoculated plots, e.g. *Centaurea stoebe* s.l. and *Artemisia campestris*. The high target-species cover reported on the donor site is correlated with a very low quantity and cover of non-target species. Once again, time will probably play a crucial role in enhancing target-species cover especially of phanerogams. For enhanced target bryophyte cover the grazing regime has to be adapted (see section 2.5.3 below).

2.5.3 Role of zoochory

Another dispersal vector in our study area consisted of the grazing donkeys. Only a fraction of species recorded on the study sites were detected epi- or endozoochorously. Epizoochory samples showed seasonal variation in species composition as described by Couvreur et al. (2004). The proportion of target species (both in terms of species and seeds) was highest at the first sampling date (June) and declined to the last. A considerable quantity of target species in dry sandy grasslands are therophytes, flowering in spring and though have ripened seeds in early summer. Additionally, the target species with highest abundance, *M. minima*, is adapted to epizoochorous dispersal with its hooked appendages (Wessels et al. 2008). Bryophyte target species, e.g. *T. ruraliformis*, can be dispersed during the whole grazing period by thallus fragments. Bryophytes and lichens are a relevant part of calcareous sandy grassland; nevertheless, only a few studies reported epizoochorous cryptogam dispersal so far (Pauliuk et al. 2011). The high ratio of ruderal species at the latter sampling dates partly reflected the dominant species in the standing vegetation. Even though the most abundant ruderal species were not explicitly adapted to epizoochory, a relatively high frequency in the vegetation at high seed production rates enabled high detection rates.

Dung of free-ranging herbivores is an important dispersal agent for various plant species both in terms of quantity of seeds and of dispersed species (Mouissie et al. 2005a). In our study only about 10 % of species recorded in the actual vegetation were detected in the dung samples, whereas in other studies around one-quarter (Cosyns et al. 2005; horse, cattle) to one-third (Stroh et al. 2012; horse) of species present in the background vegetation were recorded in dung. Because our dung samples were collected only at a single sampling date, a prolonged sampling period would presumably have had enhanced the number of detected species and thus have displayed the overall endozoochorous dispersal potential more accurately. A study conducted in a dune system by Bakker et al. (2008) revealed a maximum of species (and seed density) detected in cattle dung in July to September. Nevertheless, a high proportion of target species found in the dung samples is remarkable and the sampling date may have contributed to this. At least for two target species recorded in endozoochory samples, establishment next to dung accumulations was observed on the restoration sites (personal observation). These species, *S. conica* and *P. arenarium*, certainly originated from the inoculation material and were only recorded outside of inoculated plots since the second to third study year. The high proportion of open ground on the restoration sites may contribute to improved establishment following dispersal (see Cosyns et al. 2006).

The complementarity of epi- and endozoochory, as described by Couvreur et al. (2005a), could be observed as well; the two zoochory pathways had a few species in common and complemented each other in the majority of species.

2.5.4 Grazing effects

After four study years grazing had effects on vegetation cover of the restoration sites mainly by maintaining a high proportion of open ground and hampering the establishment of a bryophyte layer (mainly acrocarpous bryophytes). The reduction of a pleurocarpous moss, forming dense mats in later successional stages of sandy grassland, was observed by Stroh (2006) and Eichberg et al. (2010) and evaluated positively. However, on inoculated plots grazing led to a severe reduction of target bryophyte cover and thus had a negative effect on total cover of target species. We infer from this that the grazing period was too long and/or the stocking density was too high. In the case of our restoration sites the sandy substrate, which is not really solid, has to be taken into account; the donkeys always sank in a bit while grazing. Especially on frequently trampled sites this might also reduce the establishment success of seedlings.

Effects on vegetation composition like enhanced species diversity or facilitation of desired species (Rasran et al. 2007; Plassmann et al. 2010) could so far not be observed. In later successional stages opening of a dense sward and the creation of gaps probably have a positive effect on diversity of species and habitats, but in our initial successional stage gaps for establishment are not a limiting factor. An effect of grazing on the quantity of target species or their proportion of total species number was not detected. Additionally, grazing did not result in enhanced cover of target species in relation to total cover as described by Eichberg et al. (2010). A reduction of (high-growing) ruderal species as reported by Stroh et al. (2007; for sheep) could not be ascertained. In contrast, two dominant ruderal species, *Oenothera biennis* agg. (S1) and *Melilotus albus* (S2), were only slightly grazed by donkeys, which prefer a diet of graminoids (Cosyns et al. 2001). In contrast, sheep are known to graze these species preferentially (Stroh et al. 2002), therefore a mixed grazing management might have been useful to reduce ruderal species more intensively.

2.6 Concluding remarks

Our results show that, on the abiotic site, the deposition of deep sand can be used as an effective restoration measure in sand ecosystems to reduce soil nutrients and undesired seed bank species. On the biotic site, we could show that using only small amounts of plant material applied in distributed patches can be a very useful tool to overcome seed limitation and establish source or ´starter´ populations for target species in restoration projects. Grazing by donkeys should be carefully applied with respect to trampling, but has the potential to disperse high proportions of target species when grazing management is planned according to the peak season of target species´ seed ripening.

Appendix 2.1: Presence table for all species growing on the different treatment plots of the two restoration sites (n = 4; the number of plots with the species is given) in the years 2010 to 2013. Target species are highlighted in red; red list species (Germany; Korneck et al. 1996) are highlighted in dark red. Additionally, the occurrence of the species on the grid plots, in seed bank and seed rain of the two sites and in the inoculation material is given. Records of epi- and endozoochorous samples are added.

	Site 1																Site 2																grid plots S1	grid plots S2	seed bank S1	seed bank S2	seed rain S1	seed rain S2	inoc. material	endozoochory	epizoochory
	2010				2011				2012				2013				2010				2011				2012				2013												
inoculated	-		+		-		+		-		+		-		+		-		+		-		+		-		+		-		+										
grazed	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+									
Erodium cicutarium	3	1	3	1	4	2	4	2	3	3	2	2	3	3	2	1	4	3	4	3	3	2	3	3	2	1	1	2	3			2	x	x							
Corynephorus canescens	2	1	2	2	2	1	4	3	2	2	4	2	2	2	4	2		1	4	4		1	4	2		1	4	3	1	1	4	2	x	x			x		x		
Tortula ruraliformis			4	4	2	2	4	4	3	2	4	4	3	3	4	4	2	1	4	4	4		4	4	4	3	4	4	3	4	4	4	x	x							x
Rumex acetosella s.l.				2				3	2	1	1	3	2	1	2	3	3	2	2	4	4	2	2	4	4	3	4	4	4	3	4	4	x	x		x	x		x	x	
Arenaria serpyllifolia agg.			2	1			4	4		1	4	4		1	4	4		2	4	3	1	3	4	4	2	4	4	4	4	4	4	4	x	x					x	x	x
Cerastium semidecandrum							4	3			2	2	1	1	1	3		1	2	1	2	2	4	4	2	4	3	3	4	4	4	4	x	x				x	x	x	
Echium vulgare			3	4			4	4			3	3		1	2	3	3	3	4	3	3	4	4	4	1			2	2	1	1	1	x	x					x		
Medicago minima							4	4			4	4	1		4	4		1	2	1	3	1	4	4	2	2	4	4	3	3	4	4	x	x				x	x		x
Medicago lupulina															1		4	4	4	4	4	4	4	4	4	4	2	4	4	4	1	4	x	x		x				x	x
Trifolium arvense																	2	2	1	3	4	4	4	2	3	2	1	4	3	4	3	3	x	x							
Vicia angustifolia																	1	2	1	2	1		1	2	4	2	1	2	4	3	2	3	x	x				x			
Trifolium campestre															1						2	1	3	2	1	1	1	1	2	1	1	2	x	x							
Erophila verna							4	1										2			2	3	3	3		2		2	2	4		3	x	x				x	x		
Vicia lathyroides																		1		1	1	1		2		1	1	2	1	1	1	1	x	x				x	x		
Myosotis ramosissima																				1	1	1	1	2		1		1	2	3			x	x							
Valerianella locusta																												1	1				x	x							
Sanguisorba minor									3		1		3		1																										
Euphorbia cyparissias												1				1	1										1			1			x								
Veronica verna							1	1														1		1			1	1					x					x			
Cetraria aculeata							2	1			1	1	1		1												1				2									
Potentilla argentea agg.																			3	3			4	2		1	4	3		1	3	3		x				x	x	
Saxifraga tridactylites							3	3			1					1					1		4	3				2			1	1		x		x		x		
Holosteum umbellatum							3	4							2	1						1	2	1			1	1	3	3	3	1	x	x				x			
Vulpia myuros			1	3			1				1	3			1	3			3	1			3	2		1	4	3		1	3	3	x	x				x			
Phleum arenarium			4	4			4	4		1	4	4	1		4	4			4	4			4	4			4	4	1	1	4	4	x	x				x	x		
Koeleria glauca			4	4			4	4			4	4	1		4	4			4	4			4	4	1		4	4	2	1	4	4	x	x				x			
Centaurea stoebe			4	4			4	4			4	4			4	3	1		4	4	2		4	4	2		4	4	2		4	4		x				x			
Petrorhagia prolifera			1	1	1		4	3			3	2			2	2			2	1	1		4	3	1		3	3	1		3	3	x	x				x			
Silene conica			1	2			4	4			4	4			4	4			2	2			4	4			2	3		1	4	3	x	x				x	x	x	
Artemisia campestris			2	2			2	3			3	3			4	3			4	4			4	4			4	4			4	4						x			
Helichrysum arenarium			2	2			2	2			3	2			3	2			2	4			4	4			4	4			4	4	x					x			
Koeleria macrantha			1	3			3	3			1	3			1	3			1	1			3	1			3	2			3	1	x					x			
Sedum acre			3	2			1	2			2	2			2	1			4	3			4	2			2	2			4	2		x				x			
Ononis repens s.l.				1			1	3			4	3			4	2			3	4			3	4			3	4			4	4		x				x			
Peltigera rufescens			1	2			1	2			4				2	1							1				3	1			4	1									
Phleum phleoides							1				4	1			4	2							2	1			3	3			4	3						x			

Appendix 2.1: continued

	Site 1																				Site 2																				grid plots S1	grid plots S2	seed bank S1	seed bank S2	seed rain S1	seed rain S2	inoc. material	endozoochory	epizoochory
	2010					2011					2012					2013					2010					2011					2012					2013													
inoculated	-		+			-		+			-		+			-		+			-		+			-		+			-		+			-		+											
grazed	-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+										
Hieracium pilosella									1				3	2				3	2											1							1	1											
Herniaria glabra																								1				1																			
Thymus serpyllum																														1								1										
Mibora minima									1																																							
Veronica praecox																														1													x					
Cladonia cf. *rangiformis*																						1					1					2																
Helianthemum nummularium																											1					1							1									
Conyza canadensis	4	3	3	3		4	4	4	4		4	4	4	4		4	4	2	4		3	4	4	4		4	4	4	4		4	4	4	4		4	4	4	4		x	x		x	x	x	x	x	x
Elymus repens	2	2	1	1		2	2	2	3		2	2	2	3		1	2	2	3		3	2	2	2		3	4	4	3		3	3	4	3		4	3	4	4		x	x							
Oenothera biennis agg.	4	4	3	4		3	4	4	4		4	4	4	4		4	4	4	4		4	4	4	4		4	4	4	4		4	4	4	4		4	4	4	4		x	x			x	x	x	x	x
Setaria viridis	4	4	4	4		4	1	4	4		4	4	4	4		3	2		4		4	4	4	4		4	4	4	4		4	4	4	4		4	3	4	3		x	x		x			x		
Diplotaxis tenuifolia	2	3	4	3		2	3	4	2		1	2	2	1		1	2	2			4	4	4	4		3	4	4	4			1	2	3		1	1	1	3		x	x					x		
Bromus tectorum	2	3	4	4		4	4	4	4		4	4	4	4		4	4	4	4		1		4	4		1		4	4		3	2	4	4		4	4	4	4		x	x			x		x	x	
Chenopodium album agg.	2	3	2	2		3	4	2	3		4	4	1	4		1	2				4	4	2	1			4		4		4	4		4			2				x	x	x	x	x	x	x	x	x
Digitaria sanguinalis	2	3	3	2		1					1	2		1							4	4	3	4		2	4	2	3		3	3		3		1	3	1			x	x		x	x		x		
Artemisia vulgaris		1		1			1		2			1	1	2			1	1	2		3	4	4	4		2	4	4	3		4	4	4	4		2	4	3	4		x	x				x			
Equisetum arvense		1					1					1					1	1			2	3	2	3		2	3	2	3		3	2	2	3		4	3	2	2		x	x							
Corispermum leptopterum		1	3	3			1	2	2			2	1	3				1	2		4	4	4	3		3	3	1	1		4	3	2	1		3	1	2			x	x		x		x			
Carex hirta							1	1				1	2				1	1			1	1		1		1	1	1	1		1	1	1	1		1	1		1		x	x					x		
Apera spica-venti									1		1					1	1	1			1	2		2		2	3		3		2	2	2	3		3	2	1			x	x				x			
Papaver dubium s.l.						1						2					1				1	1		2		1	1	1	2		2	3		1		2	1				x	x				x		x	
Papaver rhoeas							1	1				1					1				4	4	4	3			4	1	2		2	2				1	3				x	x				x			
Convolvulus arvensis	1					2					2					2					1	1	2			1	3		1			2		1			1				x	x							
Poa angustifolia		1						3	1		3		2	3		3	1	3	3								1	2	1			2	2	2		1	2	2	3		x	x						x	
Salsola kali subsp. *Tragus*			4	4		2	2	4	4		1	2	2	4		2	2	1	4				4	4		3	3	2	3												x	x					x		
Silene latifolia subsp. *alba*								1					1					1			4	4	3	2		4	4	3	2		2	4	2	2		4	4	1	2		x	x							
Melilotus albus													1					1			4	4	3	4		4	4	3	4		4	4	4	4		4	4	4	4		x	x				x			x
Setaria pumila																					4	4	4	4		1	4	1	4		4	4	2	4		3	4	1	1		x	x		x					
Berteroa incana																					3	4	1	3		3	3	2	3		4	4		4		4	4	2	4		x	x				x			x
Eragrostis minor	1	1	4	3										2							4	4	4	4		1	2	1	1		1	1									x	x		x			x		
Robinia pseudoacacia juv.	2		1			1		1			1		1			1					1	1	3			1		2			1		2					1			x	x							
Daucus carota																					3	3	2	4		2	3	1	3		2	2		2		3	2		3			x				x			
Cirsium arvense		1		1			1		1			2	1	1			2		1			2					2					1					1				x	x			x	x			
Tanacetum vulgare			1	1				1	1				1	1				1	1		1	2	2	3		1	2	2	3		1	2	2	3			2	2	3		x	x							
Erigeron annuus								2	2				2	2				2	2		1		3	2		1	3	2	3		3	3	3	3		1	2	3	1		x	x					x		
Sisymbrium altissimum																					1	2	2	1			2		2		1	3	1	1		2	2		1		x	x				x			x
Polygonum aviculare agg.		1							1												4	4	4	4		3		2			1	2		2			1				x	x		x					
Senecio inaequidens			1	1													1				1	2	1	2			1	1				1			1	1					x	x				x			
Vicia villosa s.l.																					1	1	1	2		2	1	1			2		1			3	1	2				x							
Arabidopsis thaliana																						1		1		1	3	1	3		1	2		2		2	2				x	x							

Appendix 2.1: continued

	Site 1																				Site 2																				grid plots S1	grid plots S2	seed bank S1	seed bank S2	seed rain S1	seed rain S2	inoc. material	endozoochory	epizoochory
	2010					2011					2012					2013					2010					2011					2012					2013													
inoculated	-		+			-		+			-		+			-		+			-		+			-		+			-		+			-		+											
grazed	-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+										
Fallopia convolvulus																					4	4	2	4			2		1		1	2				3	2			x	x							
Viola arvensis												1									2	3	1	1							1	1				2	1			x	x				x			
Linaria vulgaris							1					1					1					1	3	3		1		2	1			1	1	2								x						
Lactuca serriola	2	1	1	2		1	2	1	1												1	3		2																x	x		x	x			x	
Vicia hirsuta																					1		1	1		3					1	1		2		2	1	2	1		x	x				x			
Poa compressa							1					1					1						4	3			1	4	4				4	4			1	4	4		x	x				x	x		
Veronica arvensis								2	1													1	1	1			1		1			3		3		1	3	1	2			x		x			x		
Solidago gigantea																											1	4	3		1	2	4	3		1	1	4	3			x			x	x			
Oxalis dillenii																					1	1	1	1		1			1			1		1			1					x				x			
Chenopodium strictum	4	4	4	4																	4	4	4	4			1														x	x		x					
Bromus hordeaceus		1				1						1	2				1						1										1					1	1		x	x							
Senecio vulgaris						1	2	2	2												1					2	1	2	1													x	x				x			
Calamagrostis epigejos							1					1					1														1		1			1		2	1			x							
Ballota nigra																						1	1			1	1					1					1					x							
Cirsium vulgare						1						1		1			1									1																x	x			x				
Digitaria ischaemum																															2	2		3								x	x							
Chaenorhinum minus																					1		3																					x							
Cardamine hirsuta																											1		1															x	x			x				
Sonchus asper																								1			1															x	x			x		x		
Tragopogon dubius														1																								1												
Echinochloa crus-galli												1									3	3	2	4								1									x	x							x
Lamium amplexicaule																										1	1		1			1		1		1						x							
Sonchus oleraceus	1						1														2		3	3																	x	x		x		x	x		
Veronica hederifolia																						2		2			3		2			1									x	x							
Sisymbrium officinale		1																				2	1	1																									
Persicaria maculosa																					4	4	3	4																		x							x
Atriplex sagittata																					4	4	3	3			3		1			2									x	x				x			
Anagallis arvensis																					2	2	3	2														1		1		x							
Stellaria media																										3	3	1	2									1		1	x	x					x		
Tripleurospermum perforatum		1																			2	3	2	2										1								x							
Senecio cf. *vernalis*																							1	1		1	1	1															x			x				
Bromus sterilis												1		1					1			1										1		1				3		1	x	x							
Amaranthus retroflexus		1	2	2																	3	1	2	3																		x	x	x				x		
Tussilago farfara							1		1			1												2				2						1									x			x	x			
Solanum nigrum	3	1	1	2																	4	4	4	3				1	2													x	x			x	x			
Robinia pseudoacacia seedl.																			1									1																					
Malva alcea																																		1						1		x								
Epilobium brachycarpum																								1																		x							
Panicum miliaceum																								1																									
Veronica cf. *agrestis*																												1																					
Saponaria officinalis													1					1																								x							

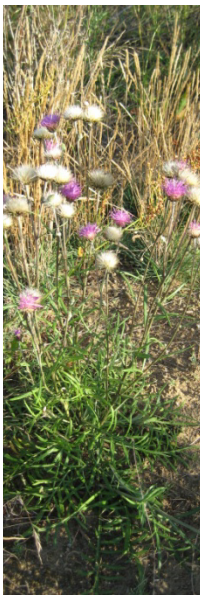
Appendix 2.1: continued

	Site 1																Site 2																grid plots S1	grid plots S2	seed bank S1	seed bank S2	seed rain S1	seed rain S2	inoc. material	endozoochory	epizoochory
	2010				2011				2012				2013				2010				2011				2012				2013												
inoculated	-		+		-		+		-		+		-		+		-		+		-		+		-		+		-		+										
grazed	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+									
Capsella bursa-pastoris																						1				2			1	1			x	x							
Carduus crispus																	1					1												x							
Datura stramonium																	2	1																x							
Reseda luteola																					1	1																			
Papaver argemone																						1				1				1				x							
Descurainia sophia																						1																			
Epilobium parviflorum														1																											
Galium aparine																						2																			
Sonchus arvensis		1																																							
Urtica dioica																														1				x		x			x		
Ambrosia artemisiifolia																	1				1				1				1					x							
Alliaria petiolata																					1													x							
Solanum physalifolium																	2																	x		x					
Bryum argenteum		1	2		4	3	4	2	4	4	4	2	4	4	4	2		1	1		4	4	4	4	4	4	4	4	4	4	4	3	x	x							
Hypochaeris radicata					1	3	4	3	1	2	4	3	1	2	3	3		1			2	3	2	3	2	2	2	4	2	4	2	4	x	x			x	x			
Prunus serotina juv.	1	1			2			1		1	2				1		1	1			2	1			2	2			2	2			x								
Holcus lanatus					2	2	3	3	1	1	1	2		1	2	2			1	2	1	1			1	1		1	1	2			x	x			x				
Pinus sylvestris juv.	3	4	3	4	2		2		3		2		3		3		4	2	4	4	2		1		2		3		3		2		x	x							
Verbascum phlomoides			2	3			3	3			2	3			1	2	1	2	4	4	1	1	4	4	1	2	4	4	4	4	4	4	x	x				x	x		
Hypericum perforatum												1				1	1	1	3	2	1	1	3	2	1	1	2	2	1	1	2	1		x		x			x		
Plantago lanceolata																	2	3	3	2	2	3	3	4	2	3	1	4	2	3	3	3		x							
Agrostis capillaris											1	1	1	1	1	2					1			2	2	1	1	3	2	2	1	4	x	x							
Cornus sanguinea																	1	1	1		1	1			1				1	2			x								
Pinus sylvestris seedl.					1		3	1					2	1	2	1					3		3	2	1	2		1				1					x	x			
Achillea millefolium										1				1				1		1		1	1	1		1	1	1		1	1	1	x	x							
Salix spec. juv.	1	2		1	1		1	1	1				1									1											x	x			x	x	x		
Festuca cf. *rubra*			2	2			3	2		1	3	2	3	1	4			1	1	2		1	2	3																	
Brachythecium albicans								1			3	3	1	1	4	4									2		4	4	2	2	4	4	x	x							
Festuca ovina agg.									3	1			1	1	1	2							1				3	2		1	2	2	x								
Senecio jacobea		1				1				2				1		1																	x								
Vicia sepium																	1				2		2											x							
Galinsoga parviflora																	1		2															x							
Betula pendula seedl.														1		1																	x	x			x	x	x		x
Quercus robur seedl.														1	1	1																									
Ornithopus perpusillus																	2			1			1											x							
Lepidium densiflorum																		1		1		1		1		1		1		1				x							
Humulus lupulus										1							1		1	2		1						1					x	x				x			
Plantago major subsp. *intermedia*				1															2	1		1		1										x						x	
Crepis capillaris							1	1										1		1		1		1		1		2		2		2	x	x							
Trifolium repens																		2	2	4		3	3	4		2		3		1		3		x							

Appendix 2.1: continued

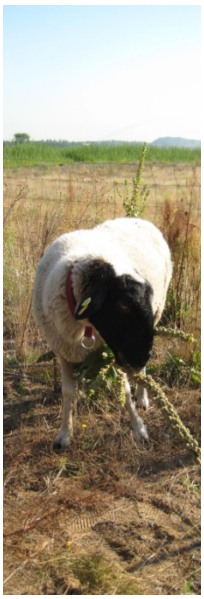
	Site 1																				Site 2																				grid plots S1	grid plots S2	seed bank S1	seed bank S2	seed rain S1	seed rain S2	inoc. material	endozoochory	epizoochory
	2010					2011					2012					2013					2010					2011					2012					2013													
inoculated	-		+			-		+			-		+			-		+			-		+			-		+			-		+			-		+											
grazed	-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+		-	+	-	+										
Rumex thyrsiflorus														1			1		1					1			1					1					1			1			x			x	
Quercus robur juv.				1										1		1			1				1				1					1							1			x							
Hypnum cupressiforme			4	4				4	4				4	3				4	4		2		4	4				1	3		1		4	3		1	1	4	3		x	x							
Cladonia cf. *pyxidata/rei*			2	2				4	3				3	1				1	1				4	3				4					3	1				2										
Dactylis glomerata				1					1														1	2					1					1						x	x							
Cladonia cf. *furcata*			2	1																			1	1																						
Secale cereale									1														2	2																						
Geranium molle																								1					1					1					2		x						
Melilotus officinalis																								1					1												x						
Acer pseudoplatanus juv.				1																																					x							
Corylus avellana																							1				1					1					1											
Sedum telephium																							1				1																				
Phragmites australis																																							1				x	x				
Rubus spec.	1					1					1													1					1					1					1			x			x				
Poa annua																						1					3					1	3							x	x							x
Claytonia perfoliata																							1									1									x							
Arrhenatherum elatius											1					2																										x							
Lolium perenne							1																1																		x							
Populus spec.		1					1																																		x	x			x	x			
Acer negundo																						1																										
Rosa spec.																																										x							
Cytisus scoparius juv.	2					2					2					2																										x							
Vicia cracca																					1																												
Acrocarpi	3	4	4	4		3	3	4	2		4	4	4	2		4	4	4	2		3	2	4	4		4	4	4	4		4	4	4	4		4	4	4	4										
Taraxacum sect. Ruderalia	3		2	1		3	2	3			2	2	1	2		3	3	2	3					1	2	1	3			1			3				2		3	x	x		x	x	x	x		
Nostoc spec.			2	2		1		4	4				4	4				4	4			1	4	4		2		4	2		2	2	4	4		1	1	3	2									
Crepis spec.																		1																														

3 Seed addition via epizoochorous dispersal in restoration: an experimental approach mimicking the colonization of bare-soil patches



Jurinea cyanoides

Experimental area 3 on ´Streitgewann´ with *Rumex thyrsiflorus* and flowering *Alyssum montanum* subsp. *gmelinii* (yellow) in 2009



restorative grazing

3.1 Abstract

Questions: Does epizoochorous dispersal via sheep lead to the establishment of populations of sandy grassland species on newly created, managed restoration sites on sandy bare soil? Do epizoochorously-induced spatial patterns persist during vegetation development? Does sandy grassland develop, which is rich in epizoochorously dispersed target species?

Methods: A six-year experiment on epizoochorous dispersal by sheep was conducted on three newly created deep sand deposition sites mimicking restoration areas with reduced nutrient availability. Establishment success and persistence of ten epizoochorously dispersed species were assessed and spatial patterns were analysed by the use of SADIE (Spatial Analysis by Distance Indices). Vegetation development of the experimental areas was related to a nearby nature reserve (relevés, target species ratios). In addition, seed rain and early-successional soil seed bank were sampled.

Results: All except one species dispersed by sheep became established and persisted during the six-year study. After establishment, most perennials did not change or increased in abundance over time, whereas annuals showed various population dynamics. Spatial patterns were aggregated for most study species. Similarity of spatial patterns between consecutive years varied by species, site and year and was stronger in perennial than in annual species. Patterns of seed dispersal and establishment were positively associated with each other (a subset of three species was tested). NMDS revealed similarities in the three experimental areas, which was related to the vegetation of the surrounding grassland. Within six years, the proportion but not the cover of target species in the experimental areas reached a level similar to that of a nearby nature reserve; however, many species characteristic of the nature reserve were absent. The species compositions of both seed bank and seed rain were dominated by non-target species.

Conclusions: Sheep flocks may assist the colonization of grassland species on newly created bare soil areas via epizoochory. The incorporation of livestock into restoration projects might facilitate the regeneration and preservation of threatened plant populations. Livestock might be most successful in promoting biodiversity if they are moved from communities with target species to restoration areas.

3.2 Introduction

Loss and fragmentation of semi-natural grasslands are major threats to populations of rare plant species in the Central European landscape (Fischer & Matthies 1998; Kéry et al. 2000). Due to land-use changes such as agricultural intensification and afforestation, the connectivity between grassland fragments decreased dramatically during the 20th Century (Soons et al. 2005; Hooftman & Bullock 2012). Therefore, restoration approaches are required that enlarge existing semi-natural grasslands and develop degraded areas as ´stepping stones´ to connect existing habitats. Successful restoration of grassland habitats includes three major aspects: abiotic restoration, biotic restoration and follow-up management. Nutrient adjustments to soil can be achieved by either removing or replacing topsoil depending on the specific site conditions and cost considerations (Hölzel & Otte 2003; Eichberg et al. 2010).

The availability of viable seeds for the restoration of target plant species may be limited by distance to well-developed plant communities and impoverished soil seed banks (Bakker & Berendse 1999). Additionally, the possibility that the regional species pool will act as a seed source for local colonization is limited by the dramatic decrease in the number of formerly widespread roaming livestock herds (´dispersal infrastructure´) in the European landscape (Ozinga et al. 2009). Effective tools to overcome seed limitations in degraded grasslands include the transfer of seed-containing plant material and sowing of seed mixtures onto bare or disturbed soil (Kiehl et al. 2010; Coiffait-Gombault et al. 2012; Hölzel et al. 2012). However, seed transfer is normally employed only at the beginning of a restoration project and, as yet, little is known regarding the long-term success of anthropogenic seed transfer. Moreover, commercial seed mixtures are often dominated by seeds of graminoids and contain high amounts of perennial generalists, which can establish and spread quickly (Conrad & Tischew 2011). Seed mixtures of local provenance provide better results with regards to target species establishment in restored communities (Mitchley et al. 2012), so that local hay or raked plant material can be used (Stroh et al. 2007; Klimkowska et al. 2010).

Livestock transport a wide range of plant species by epi- and endozoochorous seed dispersal. In this study, we focused on epizoochory, but endozoochory plays an important role as well (e.g. Eichberg et al. 2007). With respect to epizoochory, seeds are dispersed by various livestock species, e.g. equids (Couvreur et al. 2004; Couvreur et al. 2005a), cattle (Couvreur et al. 2004), goats (Shmida & Ellner 1983) and sheep (Fischer et al. 1996; Mouissie et al. 2005b; Manzano & Malo 2006; Wessels et al. 2008). Due to their curly and greasy hair, specifically sheep have a very high epizoochorous dispersal potential. In sandy grasslands,

roaming sheep flocks can disperse large numbers of seeds of many species (Wessels et al. 2008). Even though seed morphology and seed mass influence the attachment and detachment rates (Tackenberg et al. 2006; Will et al. 2007), epizoochory is possible for most if not all grassland species (Couvreur et al. 2004; Mouissie et al. 2005b).

Grazing animals can provide effective long-distance epizoochorous seed transport (Boulanger et al. 2011; Purschke et al. 2012). Boulanger et al. (2011) demonstrated that epizoochory by deer led to a widened spatial distribution of the forest herb, *Cynoglossum germanicum*, which was rare in a region of France. In Swedish grasslands with a long history of continuous grazing management by livestock, plant species with a propensity for long-distance dispersal via wind and grazing animals were well represented (Purschke et al. 2012).

Domestic livestock species can serve as very effective management tools for increasing species richness, if the livestock are managed carefully (reviewed in Rosenthal et al. 2012). Reintroduction of livestock is an important way to overcome seed limitation and provide connectivity between semi-natural grasslands (Poschlod et al. 1998; Beinlich & Plachter 2010; Auffret et al. 2012). There is much evidence that livestock moving through the landscape can provide an effective source of seeds to certain ecosystems, but the mechanisms and quantities of zoochorous colonization are not well understood (Rosenthal et al. 2012).

Experimental studies on the effectiveness of epizoochorous dispersal by livestock have dealt only with the short-term establishment success of dispersed species (Bugla 2009; Wessels-de Wit & Schwabe 2010). Eichberg et al. (2005) and Wessels-de Wit & Schwabe (2010) studied the spatial distribution of the dispersed seeds, resulting in non-random seed shadows. A better understanding of spatial patterns and their consequences for community development will help to direct habitat management (Nathan & Muller-Landau 2000). Here we present results of a long-term experiment, originally started by Wessels-de Wit & Schwabe (2010). The main objectives of our study were to assess the establishment success and spatial distribution of sheep-epizoochorously dispersed seeds over a period of six years. The following questions were addressed in our study:

(1) Do epizoochorously-dispersed plant species establish and persist in restored communities?
(2) Do the spatial patterns related to epizoochory persist in restored communities?
(3) What is the structure of the plant community developing on sheep-affected bare-soil sites in terms of species composition, vegetation cover, target species ratios and turnover ratio? Does the structure of the restored community after epizoochory by sheep correspond to the target community in nature protection areas?

3.3 Methods

3.3.1 Study area

The experiment in our study was carried out in an ex-arable sand field in the northern Upper Rhine Valley, Germany (5.5 ha; 8°34' E, 49°50' N; 101 m a.s.l.). This site was used as a field until summer 2004 and, since then, managed by restorative sheep grazing to develop a new habitat for sandy-grassland species (Koelerio-Corynephoretea). In autumn 2004, the site was inoculated once with small amounts of mown plant material from a nearby nature reserve. In 2005, three experimental areas were established by depositing nutrient-poor deep sand on this restoration site. Each sand-deposition area was 300 m^2 in extent and raised 0.7 m above the former soil surface. The areas were arranged along a line about 40 to 50 m apart. The sand material was from a construction site. The soil conditions were suitable for sand grassland restoration (pH = 7.7, N_{total} = 0.01 mg g^{-1}, phosphate-P = 9.6 mg kg^{-1}; Wessels-de Wit & Schwabe 2010). On each sand-deposition area, an individually fenced experimental area was installed, comprising 81 1-m^2 plots arranged in a nine by nine meter grid. Until 2010, fencing protected the experimental areas against uncontrolled grazing by large sheep flocks and rabbits. As a follow-up management, grazing by means of a small sheep flock was applied to the experimental areas to control ruderal species. In midsummer during 2007-2009, four sheep were present on each experimental area on one day $year^{-1}$ for approx. 4-12 hours, depending on the amount of available forage. In 2010, the fences were dismantled and since then, the experimental areas were grazed as a unit with their surroundings by a flock of 500-800 sheep during short rotations (about one day per grazing section with each section comprising one experimental area).

3.3.2 Experimental design

In October 2005, two Rhoen sheep were present for a single 24-hr period on each experimental area. One sheep was prepared with experimentally attached seeds of 14 species typical for inland sand vegetation; the second sheep was included to increase the trampling effect and as a companion. The epizoochorously dispersed species were: *Alyssum montanum* subsp. *gmelinii*, *Armeria maritima* subsp. *elongata*, *Centaurea stoebe* s.l., *Cynoglossum officinale, Jasione montana*, *Koeleria glauca*, *Medicago minima*, *Myosotis stricta*, *Phleum arenarium*, *Scabiosa canescens*, *Silene conica*, *S. otites*, *Stipa capillata* and *Tragus racemosus.* A characterization of the study species is given in Table 3.1.

Table 3.1: Plant species investigated by Wessels-de Wit & Schwabe (2010) and in this study. Epizoochory: + = seeds detected in sheep fur by Wessels et al. (2008), - = no data. Life cycle: a = annual, p = perennial. Successional stage: p = pioneer, m = mid-successional. Target species: + = yes, - = no. RL: Red List status in Germany (Korneck et al. 1996) and Hesse (Hemm et al. 2008): 1 = critically endangered, 2 = endangered, 3 = vulnerable, V = near threatened, * = currently not threatened.

Species	Epizoochory	Life cycle	Successional stage	Target species	RL
Alyssum montanum subsp. *gmelinii*	-	p	p,m	+	2/1
Armeria maritima subsp. *elongata*	+	p	m	+	3/3
Centaurea stoebe s.l.	+	p	m	+	*/*
Cynoglossum officinale	+	p	m	-	*/*
Jasione montana	-	p	p	+	*/V
Medicago minima	+	a	p	+	3/3
Myosotis stricta	+	a	p	+	*/*
Phleum arenarium	+	a	p	+	2/3
Scabiosa canescens	-	p	m	+	3/3
Silene conica	+	a	p	+	3/3
Silene otites	+	p	p,m	+	3/3
Stipa capillata	+	p	m	+	3/3
Tragus racemosus	-	a	p	-	*/*

For most of these species, natural epizoochorous dispersal had been documented (Wessels et al. 2008) or is likely (Couvreur et al. 2004; Mouissie et al. 2005b). Seeds were collected in sandy habitats in the vicinity of the experimental site (covering an area of about 100 ha); the seeds originated from different sites and, depending on seed production, at least ten individuals. One species, *K. glauca*, was eliminated from consideration in the present study due to overlapping of the experiment presented here and a trampling experiment conducted simultaneously in 2005, in which additional seeds of this species were introduced into the experimental areas (see Wessels-de Wit & Schwabe 2010). Per body part (shoulder, flank and back on both sides of the sheep) and per plant species, 100 seeds were attached (in total, 600 seeds per species per sheep). Association of seed shadows and emerging seedlings were studied concurrently for three study species (*C. officinale, M. minima* and *S. capillata*), which can occur in high densities in sheep fur due to effective seed appendages (Wessels et al. 2008). The seeds of these species are large enough to be detected on the soil surface and these seeds also were marked with yellow dye. For further details on seed and sheep preparation, see Wessels-de Wit & Schwabe (2010). Post-dispersal seedling emergence and establishment of all introduced species were recorded until 2011.

We did not include plots where sheep were not a part of the experiment. The study site was very open so that a wind-driven seed exchange between experimental plots was likely. In the course of longer-term experiments, it is nearly impossible to exclude such effects and to avoid plot-to-plot contamination (see Lepš et al. 2007; Eichberg et al. 2010). The use of almost seed-free deep sand (see also Eichberg et al. 2010) as a substrate for plant establishment allowed the exclusion of soil as a significant plant source. Seed rain was sampled as well and only data of those epizoochorously introduced species were analysed, which were not detected in seed traps or were absent in the surrounding vegetation.

3.3.3 Vegetation relevés

Individuals of the introduced species were counted on every 1-m^2 plot over a five-year period (Nov. 2005, May/June 2006-2009). In addition, the percentage cover of the emerging vegetation was estimated on a per-plot basis (2006-2009) and in later surveys, for the entire experimental area (2009-2011), using the following scale: 0.1, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25,... 95, 100 %.

To set vegetation composition of the experimental areas in context, five 80-m^2 circular patches were sampled along a transect in the adjacent former field (inter-plot distance: 45 m) and five grid-based 80-m^2 circular patches in a nearby well-developed Koelerio-Corynephoretea sand ecosystem (nature reserve ´Griesheimer Düne und Eichwäldchen´; inter-plot distance: 50 to 120 m; aerial distance to the experimental areas approx. 300 m). Assessment of vegetation cover on these plots used the same cover scale and took place in the same time period as in the experimental areas.

3.3.4 Seed rain

Seed rain was analysed starting in the main fruiting period of the first year of community development (July 2006 to June 2007) using funnel traps (Kollmann & Götze 1998). Each fenced area had ten funnel traps arranged evenly around the experimental area (a 0.5 m buffer zone between the fence and the experimental area was used for trap installation) 0.9 m above ground level. Total sampling area per experimental area was 0.452 m^2. Traps were emptied fortnightly. Vegetation surrounding the traps was cut regularly at ground level within a radius of approx. 0.5 m to avoid direct seed input into the traps. Trapped seeds were

identified and counted; determination was conducted by means of a reference seed collection and literature (Beijerinck 1976; Berggren 1981; Anderberg 1994; Cappers et al. 2006).

Seed rain analysis was conducted by a diploma student (Retta 2007).

3.3.5 Soil seed bank

In March 2007, one growing season after the sand was deposited, the soil seed bank of the experimental areas was sampled. Previous studies carried out in our study region have shown that freshly deposited deep sand (gathered from ≥ 1 m depth) was almost free of seeds (Eichberg et al. 2010). Per experimental area, 100 individual soil samples were taken from the outer area edges in regular intervals using an Eijkelkamp liner sampler (diameter 4.7 cm; Giesbeek, NL). The samples were subdivided into an upper (1-6 cm depth) and a lower layer (11-16 cm). Per experimental area and soil layer, ten composite samples were analysed by mixing ten individual samples, respectively (according to the method used by Eichberg et al. 2006).

A seedling emergence method (Eichberg et al. 2006) was used to assess seed contents. Prior to outdoor exposure in the botanical garden of the ´Technische Universität Darmstadt´, the sand samples were sieved (mesh width: 5 mm), filled into trays and dried at room temperature for six weeks to eliminate vegetative propagules. Samples were placed on a transparently-roofed platform (0.9 m height) and covered by gauze as a protection against anemochorous seed input. As a control for aerial seed contamination, trays with autoclaved sand were positioned between the samples. The sand was kept moist and turned every second month. From May 2007 to Nov. 2008, emerging seedlings were determined, counted and removed.

Seed bank sampling and the first part of seedling assessment in 2007 were conducted by a diploma student (Retta 2007).

3.3.6 Data analysis

Spatial patterns of the introduced species on the experimental areas were studied for the time period 2006-2009 by the use of SADIE (Spatial Analysis by Distance Indices; Perry 1998). For the SADIE approach, we excluded three species (*C. stoebe* s.l., *M. stricta*, *S. conica*) because we detected these species in the seed rain traps; therefore, seeds of these species might have

reached the experimental areas from the surrounding grasslands. For all other species, seed dispersal did not come from external sources. These species were either not present in the surrounding vegetation or the seeds were detected on the ground directly after epizoochorous input.

The following variables were assessed to identify spatial patterns: i) index of aggregation I_a, ii) clustering indices (patch cluster index v_i, gap cluster index v_j) and iii) spatial association X.

i) SADIE measures the minimum total distance samples must extend to produce a completely regular arrangement (´distance to regularity´ D). The index of aggregation is defined as $I_a = D/E_a$ (with E_a: ´mean distance to regularity´). Aggregated distributions have values of $I_a > 1$, random distributions of $I_a = 1$ and regular distributions of $I_a < 1$. A formal test of spatial randomness of the observed counts among the given sample units is provided by the value P_a. A test for significance at the 5 % level indicates a regular arrangement when $P_a > 0.975$; $P_a < 0.025$ indicates aggregated distribution (Perry 1998).

ii) To identify areas of clustering, SADIE measures to which degree the sub-units contribute towards clustering. Sub-units with greater counts than the sample mean are ascribed a patch cluster index v_i (positive value); $v_i > 1.5$ is indicating patches. The gap cluster index v_j is defined similarly except that sub-units with smaller counts than the sample mean are assigned (negative values); values of $v_j < -1.5$ belong to gaps (Perry et al. 1999). The level of significance for patches and gaps is provided by the values P_i, $P_j < 0.025$, respectively.

iii) To quantify the similarity between the spatial patterns of two consecutive years and the changes of spatial structure over time, the spatial association χ is measured by comparing the clustering indices (Conrad et al. 2006). The mean of these local values χ is the overall spatial association X (Perry & Dixon 2002). Positive association is indicated by overlapping of patches or of gaps in two years, negative dissociation is indicated by coincidence of opposite forms of spatial pattern. Significance of X is tested through randomisations, with values of the cluster indices reassigned amongst the sample units, after allowance for small-scale spatial autocorrelation (Winder et al. 2001). Spatial autocorrelation in a data set can be detected in SADIE (Dutilleul 1993). A two-tailed randomisation test at the 5 % level indicates significant association if $P_a < 0.025$ and significant dissociation if $P_a > 0.975$.

For analysis of spatial patterns SADIEShell v. 1.22 (IACR-Rothamsted, UK) was used and red-blue plots (given here in black-gray) were visualised using Surfer 8.02 (Golden Software Inc., Golden, CO, USA).

Plant community structure was analysed by non-metric multidimensional scaling (NMDS) using PC-ORD 6.07 (McCune & Mefford 2011). To allow comparability vegetation data of the years 2006 to 2008 were converted from 1-m^2 recordings to a relevé including the entire 81 m^2-area. Prior to analysis, data from the vegetation cover, soil seed bank and seed rain were presence-absence transformed. As a distance measure, the Sørensen similarity index was used. Based on the stress values of initial ordinations, two dimensions were selected for the final ordination, performed with 1000 starting coordinates, 500 iterations and Monte Carlo tests on 1000 runs. The explained variance of the respective ordination axis was represented by coefficients of determination between distances in the ordination space and Sørensen distances in the original space.

Target species ratios (TSR) were calculated for the evaluation of the restoration success according to Eichberg et al. (2010) as:

TSR_{qual} = number of target plant species/total number of plant species, and
TSR_{quant} = cover sum of target plant species/cover sum of all plant species.

Denominated as target species were those from the classes Festuco-Brometea and Koelerio-Corynephoretea.

The turnover ratio was assessed as the percentage of exchanged plant species between two consecutive years.

Mixed linear models (SAS 9.2, PROC GLIMMIX; SAS Institute Inc., Cary, NC, USA; Littell et al. 2006) were applied to calculate 1) the effect of ´year´ on individual numbers of the study species (2005-2009) and 2) the effects of variables ´year´ and ´area´ (experimental areas and nature reserve) as well as their interaction effect on both target species ratios (2006-2011). Individual numbers were log(x+1)-transformed beforehand and a hypothetic zero-value (Sept. 2005) prior to epizoochorous seed input was included. Also in this case, we excluded the above-mentioned three species (see SADIE).

Mixed linear models are suitable for analysis of repeated-measures data (Littell et al. 1998). According to the corrected Akaike information criterion (AICC), 14 covariance structures were compared (Fernández 2007). If equal AICC values were assessed by two covariance structures, the simpler structure was chosen. Degrees of freedom were calculated using the Kenward-Roger approximation (Schaalje et al. 2002). For estimation of the model parameters the restricted maximum likelihood method was used. Studentised residuals and conditional studentised residuals were examined for normality using a graphical display (histograms and quantile-residuum plots); a nearly Gaussian distribution could be ascertained. Post-hoc tests

with adjustment by simulation tests were carried out to determine differences between years (significance level $\alpha = 0.05$). In cases with significant interactions, the SLICE option in the LSMEANS statement was used to assess differences between the areas for each year (Schabenberger et al. 2000).

3.4 Results

3.4.1 Establishment and persistence

All species which had been manually attached to sheep established seedlings after epizoochorous dispersal (Table 3.2). Six years after the experiment had started, nine of ten species persisted on the experimental areas. These species varied in their establishment and persistence rates. Year had a significant effect on the density of all study species except *J. montana* and *T. racemosus*. Three hemicryptophyte perennials significantly increased in density until 2009, even though many individuals were immature. The other three hemicryptophytes decreased (*S. capillata*, *S. canescens* and *J. montana*; Table 3.2). Annuals showed various population dynamics. All study species had low mean cover (≤ 1 %) in 2011.

One species, *A. montanum* subsp. *gmelinii*, spread successfully into the surrounding area after dispersal by sheep. Individuals of this species originally did not occur in the adjacent vegetation.

Table 3.2: Mean density of individuals per species and per m^2 (± SE; n = 3) between Sept. 2005 (hypothetic zero-value before the experiment started) and May/June 2009 and cover data for 2009 to 2011 (per entire experimental area of 81 m^2; mean ± SE; n = 3). Mixed linear models were calculated for 2005 to 2009; different letters indicate significant differences between years at = 0.05 (simulation tests). ´df num´ = degrees of freedom numerator, and ´df de´ = degrees of freedom denominator. Experimental areas were grazed since 2007 (2007-2009: four sheep, 2010-2011: flock of 500-800 sheep).

					Number of individuals m^{-2}						Cover (%)		
Species	df num	df de	*F*	*α*	Sept. 2005	Nov. 2005	2006	2007	2008	2009	2009	2010	2011
*Alyssum * gmelinii*	5	9.17	40.36	<.0001	$0^{a} \pm 0$	$1.3^{b} \pm <.05$	$1.6^{bd} \pm 0.1$	$12.5^{bcd} \pm 6.6$	$10.0^{cd} \pm 39$	$12.8^{c} \pm 4.4$	0.7 ± 0.3	0.7 ± 0.6	0.1 ± <.05
*Armeria * elongata*	5	9.491	15.34	0.0003	$0^{a} \pm 0$	$0.5^{b} \pm 0.2$	$0.4^{b} \pm 0.1$	$0.2^{b} \pm 0.1$	$2.1^{b} \pm 1.7$	$1.6^{b} \pm 1.2$	0.1 ± <.05	0.4 ± 0.3	0.4 ± 0.3
Cynoglossum officinale	5	9.06	92.87	<.0001	$0^{a} \pm 0$	$<.05^{a} \pm <.05$	$2.3^{b} \pm 0.5$	$1.5^{b} \pm 0.4$	$15.3^{c} \pm 5.9$	$16.3^{c} \pm 6.7$	0.1 ± <.05	0.1 ± <.05	0.1 ± <.05
Jasione montana	5	12	0.84	0.5464	0 ± 0	<.05 ± <.05	<.05 ± <.05	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Medicago minima	5	9.06	92.87	<.0001	$0^{a} \pm 0$	$0.3^{b} \pm 0.1$	$0.6^{b} \pm 0.1$	$0.8^{b} \pm 0.2$	$6.8^{bc} \pm 5.8$	$14.1^{c} \pm 8.8$	1 ± 1	4 ± 1	1 ± 0
Phleum arenarium	5	12	91.96	<.0001	$0^{a} \pm 0$	$0.1^{b} \pm 0.1$	$1.4^{c} \pm 0.1$	$36.3^{d} \pm 10.1$	$29.8^{d} \pm 6.3$	$18.7^{d} \pm 7.7$	0.1 ± <.05	0.1 ± 0.3	0.1 ± <.05
Scabiosa canescens	5	10	9.12	0.0017	$0^{a} \pm 0$	$0.1^{b} \pm <.05$	$<.05^{a} \pm <.05$	$0^{a} \pm 0$	$0^{a} \pm 0$	$<.05^{a} \pm <.05$	<.05 ± <.05	<.05 ± <.05	<.05 ± <.05
Silene otites	5	12	12.00	0.0002	$0^{a} \pm 0$	$0.3^{bc} \pm <.05$	$<.05^{ab} \pm <.05$	$0.1^{ab} \pm <.05$	$1.1^{bc} \pm 1.0$	$3.1^{c} \pm 0.2$	0.1 ± <.05	1.3 ± 0.3	1.0 ± 0.6
Stipa capillata	5	9.591	26.75	<.0001	$0^{a} \pm 0$	$0^{a} \pm 0$	$0.3^{b} \pm 0.1$	$0.3^{b} \pm 0.1$	$0.2^{b} \pm 0.1$	$0.2^{b} \pm 0.1$	0.4 ± 0.3	0.7 ± 0.3	0.4 ± 0.3
Tragus racemosus	5	9.659	3.31	0.0524	0 ± 0	0 ± 0	0.1 ± <.05	0.3 ± 0.2	2.1 ± 1.5	0.1 ± <.05	0.1 ± <.05	<.05 ± <.05	<.05 ± <.05

3.4.2 Seed rain

In total, 41 taxa were detected in the funnel trap samples. Non-target species had a higher species richness and density than seeds of target species (Table 3.3). Accounting for 93 % of the total amount of seeds, the neophyte species, *Conyza canadensis*, was most abundant in the aerial diaspore pool. Target species accounted for 24 % of the taxa (ten species; among them four study species; Appendix 3.1), whereas their proportion of seeds was approximately 0.5 %. *Vulpia myuros* and *Corynephorus canescens* had the highest abundances among target species. According to life-forms, the aerial seed rain was dominated by seeds of annuals (54 % of taxa, 99 % of seeds). A high proportion (78 %) of species detected in the seed rain also established as seedlings or adults on the experimental areas and/or in the surrounding vegetation. Allochthonous species were mostly woody species (e.g. *Betula pendula*).

3.4.3 Soil seed bank

One growing season after deep-sand deposition, the seed banks of the three areas were composed of 31 taxa, seven of which were target species of the Koelerio-Corynephoretea (Table 3.4). Mean total densities of seeds reached 1170 ± 217 seeds m^{-2} (mean ± SE) in the upper soil layer and 409 ± 39 seeds m^{-2} in the lower layer. Target species' seeds had a total abundance of only 7 % (both layers pooled; e.g. *Arenaria serpyllifolia* agg. and *Saxifraga tridactylites*). Among the species tested in the epizoochory experiment, only *M. minima* was detected in low density in the seed bank (two seedlings). The ruderal species, *Conyza canadensis* and *Sisymbrium altissimum*, were the most abundant species, which together comprised more than 80 % of the total seedlings. Annuals comprised 70 % of the taxa and 96 % of the seedlings. Most species present in the seed banks were also present in the standing vegetation of the experimental areas and/or the surrounding grassland.

Table 3.3: Seed rain data, collected via funnel traps (0.9 m above ground) from July 2006 to June 2007. Target species are highlighted in red, species of the epizoochory experiment are underlined. Data provided by I. Retta.

Taxa	No. of seeds m^{-2} within one year
Conyza canadensis	29912
Sisymbrium altissimum	1211
Rumex thyrsiflorus	316
Apera spica-venti	153
Verbascum phlomoides	130
Salix spec.	102
Vulpia myuros	64
Corynephorus canescens	49
Rosaceae	29
Betula pendula	27
Arrhenatherum elatius	24
Chenopodium album agg.	22
Bromus tectorum	18
Petrorhagia prolifera	18
Melilotus albus	15
Artemisia vulgaris	11
Cornus sanguinea	11
Crepis capillaris	11
Calamagrostis epigejos	9
Lactuca serriola	9
Myosotis stricta	7
Oenothera biennis agg.	7
Solanum nigrum	7
Sorbus aucuparia	7
Phleum arenarium	4
Poaceae	4
Senecio vulgaris	4
Sonchus asper	4
Amaranthus cf. *retroflexus*	2
Arenaria serpyllifolia agg.	2
Brassicaceae	2
Carpinus betulus	2
Caryophyllaceae	2
Centaurea stoebe s.l.	2
Cerastium semidecandrum	2
Chondrilla juncea	2
Helichrysum arenarium	2
Papaver dubium s.l.	2
Portulaca oleracea	2
Prunus cf. *laurocerasus*	2
Silene conica	2
indetermined	7

Table 3.4: Seed bank data of deep sand sampled 1 ½ years after deposition in March 2007. Data from experimental areas 1-3 are composed of 100 individual soil samples per layer, respectively (see section 3.3.5 ´Soil seed bank´). The number of seedlings per m^2 is given. Target species are highlighted in red, study species are underlined. SE = standard error. Data partly provided by I. Retta.

Layer	**1-6 cm**					**11-16 cm**				
Experimental area	**1**	**2**	**3**	**mean**	**SE**	**1**	**2**	**3**	**mean**	**SE**
No. of diaspores	**1089**	**841**	**1579**	**1170**	**217**	**392**	**352**	**484**	**409**	**39**
No. of species	**19**	**12**	**17**	**16**	**2**	**11**	**12**	**10**	**11**	**1**
Amaranthus retroflexus	6	0	6	4	2	6	0	0	2	2
Arenaria serpyllifolia agg.	63	35	12	37	15	29	6	0	12	9
Betula pendula	0	0	6	2	2	12	17	23	17	3
Cardamine hirsuta	0	0	6	2	2	0	0	0	0	0
Caryophyllaceae	12	0	0	4	4	0	0	0	0	0
Cerastium semidecandrum	6	12	6	8	2	0	0	0	0	0
Chenopodium album agg.	12	6	23	13	5	17	0	0	6	6
Chenopodium pumilio	12	0	0	4	4	12	0	0	4	4
Conyza canadensis	265	334	980	526	228	219	63	398	227	97
Diplotaxis tenuifolia	0	0	0	0	0	0	0	6	2	2
Epilobium spec.	12	6	6	8	2	6	0	0	2	2
Eragrostis minor	12	0	12	8	4	6	6	0	4	2
Medicago minima	0	0	6	2	2	0	6	0	2	2
Melilotus albus	6	0	0	2	2	0	75	0	25	25
Poa annua	6	0	0	2	2	0	0	0	0	0
Poaceae	0	0	6	2	2	0	0	0	0	0
Portulaca oleracea	0	0	6	2	2	0	0	0	0	0
Potentilla argentea agg.	12	0	0	4	4	0	0	12	4	4
Rumex thyrsiflorus	0	0	6	2	2	0	0	6	2	2
Salix spec.	0	6	6	4	2	0	0	0	0	0
Salsola kali	6	6	0	4	2	0	17	6	8	5
Saxifraga tridactylites	29	6	12	15	7	0	12	0	4	4
Setaria viridis	0	0	0	0	0	0	0	6	2	2
Sisymbrium altissimum	553	403	473	476	43	69	104	17	63	25
Solanum nigrum	6	6	0	4	2	0	23	0	8	8
Sonchus asper	0	6	6	4	2	0	6	6	4	2
Stellaria media	6	0	0	2	2	12	0	0	4	4
Tragus racemosus	0	0	6	2	2	0	0	0	0	0
Urtica dioica s.l.	0	0	0	0	0	0	6	0	2	2
Verbascum phlomoides	6	6	0	4	2	0	0	0	0	0
Veronica arvensis	12	0	0	4	4	0	0	0	0	0
Vicia lathyroides	0	0	0	0	0	6	0	0	2	2
Vulpia myuros	40	0	0	13	13	0	0	6	2	2
Dicots indetermined	12	12	0	8	4	0	12	0	4	4

3.4.4 Spatial patterns

In nearly all years of the study, most sheep-dispersed species showed overall aggregated spatial patterns on the three experimental areas (Table 3.5). A random spatial arrangement was recorded for six species, depending on the specific area and/or year. Three of these species had low emergence rates (*J. montana*, *S. canescens*, *T. racemosus*).

Clustering indices varied according to species, year and experimental area. Only *A. montanum* subsp. *gmelinii* had distinguishable patches and gaps in all experimental areas during the entire study (Fig. 3.1). All other species changed their aggregation pattern with respect to patches and gaps depending on year and experimental area (e.g. *C. officinale*, *S. capillata*). *Silene otites* did not form significant patches or gaps on the experimental areas until 2009.

Spatial patterns of seed shadows and seedling establishment for *C. officinale*, *M. minima* and *S. capillata* were associated with each other.

Overall spatial association *X* differed largely between study species (Table 3.6). Perennial species mostly showed association, except for *S. otites* (exp. area 3, all years). Positive association was also found for the annual *P. arenarium*. The only species showing dissociation in spatial patterns was *T. racemosus* on experimental area 3 between 2006 and 2007.

Table 3.5 (next page): Spatial patterns of established plants after epizoochorous seed dispersal between 2006 and 2009. Index of aggregation I_a shows aggregation if $I_a > 1$. v_i and v_j designate the mean clustering indices for patches ($v_i > 1.5$) and gaps ($v_j < -1.5$), respectively. Indices with a level of significance P_a, P_i, $P_j < 0.025$ are shown in bold. ´–´ = spatial patterns not calculable. Spatial patterns of seeds (Dec. 2005) of *C. officinale*, *M. minima* and *S. capillata* are given. *Centaurea stoebe* s.l., *Myosotis stricta* and *Silene conica* are eliminated because secondary seed input by wind at the beginning of the experiment cannot be excluded.

Experimental area	1			2			3		
Unit	I_a	v_i	v_j	I_a	v_i	v_j	I_a	v_i	v_j
Alyssum montanum subsp. *gmelinii*									
2006	**1.680**	**1.483**	**-1.652**	**1.756**	**1.528**	**-1.679**	**2.007**	**1.922**	**-2.024**
2007	**2.526**	**2.505**	**-2.326**	**1.805**	**1.760**	**-1.579**	**2.166**	**2.059**	**-2.179**
2008	**2.329**	**2.339**	**-2.160**	**1.848**	**1.722**	**-1.629**	**1.875**	**1.788**	**-1.766**
2009	**2.615**	**2.325**	**-2.423**	**2.036**	**1.999**	**-1.800**	**2.054**	**1.694**	**-1.964**
Armeria maritima subsp. *elongata*									
2006	1.174	1.164	-1.182	1.299	1.099	-1.305	**1.851**	**1.578**	**-1.847**
2007	**1.628**	**1.664**	**-1.626**	1.104	1.147	-1.107	1.302	**1.455**	-1.299
2008	**1.626**	**1.674**	**-1.624**	1.220	1.230	-1.210	**1.521**	**1.590**	**-1.498**
2009	**1.637**	**1.659**	**-1.638**	1.238	1.248	-1.231	1.245	1.207	-1.210
Cynoglossum officinale									
2005 (Seeds)	**1.584**	**1.417**	**-1.587**	**2.028**	**1.995**	**-1.925**	**1.789**	**1.838**	**-1.783**
2006	**1.787**	**1.432**	**-1.655**	**2.238**	**2.245**	**-2.146**	**2.184**	**1.834**	**-2.146**
2007	**1.886**	**1.648**	**-1.792**	**2.174**	**2.004**	**-1.998**	**2.020**	**1.660**	**-2.021**
2008	1.380	**1.421**	-1.377	**2.320**	**2.439**	**-2.154**	**2.830**	**2.735**	**-2.633**
2009	0.989	0.978	-0.970	**1.758**	**1.916**	**-1.699**	**2.060**	**1.875**	**-1.816**
Jasione montana									
2006	0.756	0.998	-0.734	–	–	–	–	–	–
2007	–	–	–	–	–	–	–	–	–
2008	–	–	–	–	–	–	–	–	–
2009	–	–	–	–	–	–	–	–	–
Medicago minima									
2005 (Seeds)	**1.944**	**1.901**	**-1.950**	**2.075**	**2.179**	**-2.075**	**1.504**	**1.487**	**-1.492**
2006	1.021	0.965	-1.020	**2.120**	**1.740**	**-2.118**	**2.049**	**1.800**	**-2.022**
2007	1.027	0.952	-1.017	1.212	1.108	-1.219	**2.287**	**1.994**	**-2.253**
2008	0.936	0.938	-0.919	1.208	1.107	-1.169	**2.267**	**2.384**	**-2.262**
2009	1.180	1.249	-1.114	**1.476**	**1.475**	**-1.445**	**2.267**	**2.384**	**-2.262**
Phleum arenarium									
2006	**1.746**	**1.679**	**-1.711**	**2.000**	**1.720**	**-1.899**	**2.314**	**1.937**	**-2.282**
2007	**2.503**	**2.095**	**-2.287**	**2.428**	**2.129**	**-2.110**	**2.035**	**1.884**	**-1.920**
2008	**2.659**	**2.219**	**-2.409**	**2.093**	**1.811**	**-1.792**	1.336	1.295	-1.311
2009	**2.109**	**1.709**	**-1.928**	**1.895**	**1.775**	**-1.557**	**2.116**	**1.864**	**-1.872**
Scabiosa canescens									
2006	–	–	–	–	–	–	–	–	–
2007	–	–	–	–	–	–	–	–	–
2008	–	–	–	–	–	–	–	–	–
2009	–	–	–	0.836	1.001	-0.821	–	–	–
Silene otites									
2006	0.868	0.954	-0.858	1.274	1.147	-1.272	1.013	0.889	-1.032
2007	0.935	1.107	-0.938	**1.754**	**1.733**	**-1.745**	1.076	1.013	-1.084
2008	0.979	1.184	-0.977	**1.832**	**2.283**	**-1.798**	1.015	0.899	-1.037
2009	**1.777**	**1.888**	**-1.740**	**1.959**	**2.183**	**-1.939**	**2.013**	**2.047**	**-2.007**
Stipa capillata									
2005 (Seeds)	**2.180**	**1.543**	**-2.190**	**1.976**	**2.039**	**-1.849**	**2.047**	**1.859**	**-1.843**
2006	1.975	1.872	-1.976	1.339	1.141	-1.341	1.349	1.208	-1.365
2007	2.064	1.776	-2.060	**1.405**	**1.362**	**-1.409**	1.163	1.233	-1.151
2008	1.676	1.574	-1.677	1.290	1.260	-1.292	1.324	1.303	-1.325
2009	1.656	1.559	-1.652	1.349	1.270	-1.348	1.229	1.147	-1.228
Tragus racemosus									
2006	1.043	1.109	-1.039	1.286	1.317	-1.285	1.141	1.117	-1.136
2007	–	–	–	**1.754**	**1.733**	**-1.745**	0.789	0.813	-0.773
2008	–	–	–	**1.387**	**1.483**	**-1.371**	**1.427**	1.290	**-1.395**
2009	–	–	–	1.240	1.137	-1.244	1.203	1.464	-1.202

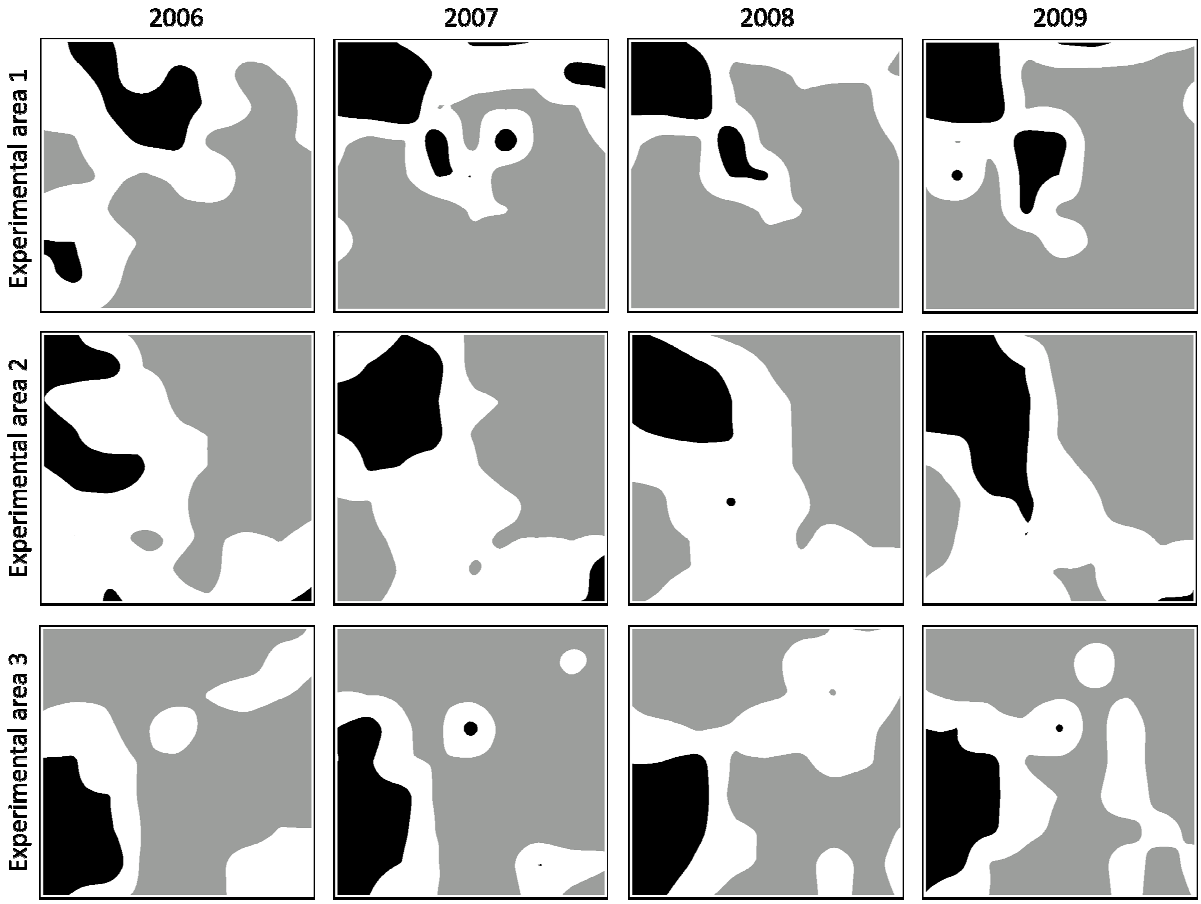


Fig. 3.1: Development of spatial patterns of *Alyssum montanum* subsp. *gmelinii* recorded in an experiment on epizoochorous seed dispersal by sheep in the period 2006-2009. Patches (v_i > 1.5) are indicated by black zones, gaps (v_j < -1.5) are coloured in gray. Only significant values are mapped (see section 3.3.6 ´Data analysis´). Each quadrat represents an area of 9 m × 9 m.

Table 3.6: Mean spatial association *X* between seed patterns (2005) and plant patterns in consecutive years (2006-2009) on the experimental areas (± SE; n = 3; *T. racemosus* n = 2). For *Jasione montana* and *Scabiosa canescens* across the whole study period association was not calculable. • = no data for calculation collected. Shown in bold are those values showing significant association on all experimental areas. Spatial association was calculated using SADIE (see section 3.3.6 ´Data analysis´).

Species	2005-06	2006-07	2007-08	2008-09
*Alyssum * gmelinii*	•	**0.68** ± 0.11	**0.80** ± 0.05	**0.83** ± 0.05
*Armeria * elongata*	•	**0.72** ± 0.05	**0.65** ± 0.17	**0.81** ± 0.12
Cynoglossum officinale	**0.67** ± 0.08	**0.83** ± 0.05	**0.33** ± 0.03	0.42 ± 0.18
Medicago minima	0.42 ± 0.18	0.29 ± 0.12	0.48 ± 0.14	**0.61** ± 0.08
Phleum arenarium	•	**0.57** ± 0.16	**0.57** ± 0.13	**0.54** ± 0.14
Silene otites	•	0.14 ± 0.06	0.53 ± 0.17	0.48 ± 0.29
Stipa capillata	**0.56** ± 0.14	**0.70** ± 0.17	**0.59** ± 0.15	**0.80** ± 0.01
Tragus racemosus	•	-0.28 ± 0.08	-0.17 ± 0.11	**0.55** ± 0.15

3.4.5 Community development

The initial vegetation of the experimental areas was dominated by ruderal species. After the first year of the study, the mean number of ruderal species declined continuously (especially early-successional Stellarietea mediae species); the total number of plant species on the experimental areas declined as well after a maximum peak in 2009 (Appendix 3.1). On the experimental areas, the mean number of target species was approximately stable; their cover increased after 2006. As a consequence, both qualitative and quantitative target species ratios of the experimental areas increased during the study period (Table 3.7); those of the nature reserve were stable (TSR_{quant}) or declining (TSR_{qual}). Until 2010, the qualitative TSR differed between both areas; in 2011, mean TSR_{qual} of the experimental areas reached the same value as the nature reserve. The TSR_{quant} was not significantly affected by year and area, even though TSR_{quant} on the experimental areas was still half the TSR_{quant} of the reserve in 2011. Total cover of target species was much higher in the target community (e.g., *S. capillata*, *Ononis repens* s.l. and *Phleum phleoides*).

NMDS ordination of the complete community-based data set revealed an increasing separation of the nature reserve from the experimental areas and the surrounding restoration site along the first axis (explained variance: 52.9 %; Fig. 3.2). The second axis separated the diaspore pools (seed rain, soil seed bank) from the vegetation recordings, and, to a lesser extent, the experimental areas from the surrounding vegetation site (explained variance: 32.7 %). Time trajectories of the experimental areas consistently showed a development following the development of the surrounding restoration site.

The similarity of species spectra of experimental areas and nature reserve plots was not very high (Appendix 3.1): 42 % of the 148 recorded species occurred on both sites, and 26 % solely in the nature reserve. With regard to target species, even 23 species (46 %) occurred only in the reserve (in high presence, e.g., *Alyssum alyssoides*, *Euphorbia seguieriana*, *O. repens*, *P. phleoides*); four species (8 %) occurred only on the experimental areas (three of these species were study species). Turnover ratios of species on the experimental areas varied between sets of years. Nearly 23 % of all species were replaced between 2006 and 2007, whereas the ratio decreased to 10 % in 2008/09 and increased again in the last year to 15 %. The nature reserve had a consistently high yearly species turnover of about 22 %, with a slight increase in turnover from 2010 to 2011.

Table 3.7: Effects of area and year on qualitative and quantitative target species ratios as tested by mixed linear models. ´df num´ = degrees of freedom numerator, ´df de´ = degrees of freedom denominator. In the right part of the table mean values of the experimental areas and the nature reserve in each year (± SE) are given. Different letters indicate significant differences between areas sliced by year at $\alpha = 0.05$ (simulation tests), only if area*year is significant.

	df num	df de	*F*	*P*	2006	2007	2008	2009	2010	2011
TSR qual					exp. area					
area	1	6.89	28.02	0.0012	0.30 [a] ± 0.02	0.32 [a] ± 0.04	0.36 [a] ± 0.02	0.36 [a] ± 0.01	0.41 [a] ± 0.02	0.44 [a] ± 0.01
year	5	28.77	0.68	0.6392	nature reserve					
area*year	5	28.77	8.30	<.0001	0.60 [b] ± 0.04	0.57 [b] ± 0.02	0.53 [b] ± 0.04	0.54 [b] ± 0.03	0.51 [b] ± 0.02	0.43 [a] ± 0.02
TSR quant					exp. area					
area	1	7.871	75.39	<.0001	0.16 ± 0.03	0.32 ± 0.03	0.36 ± 0.03	0.39 ± 0.09	0.45 ± 0.09	0.40 ± 0.11
year	5	28.2	2.24	0.0775	nature reserve					
area*year	5	28.2	1.45	0.2382	0.77 ± 0.05	0.81 ± 0.05	0.84 ± 0.03	0.78 ± 0.07	0.79 ± 0.03	0.79 ± 0.03

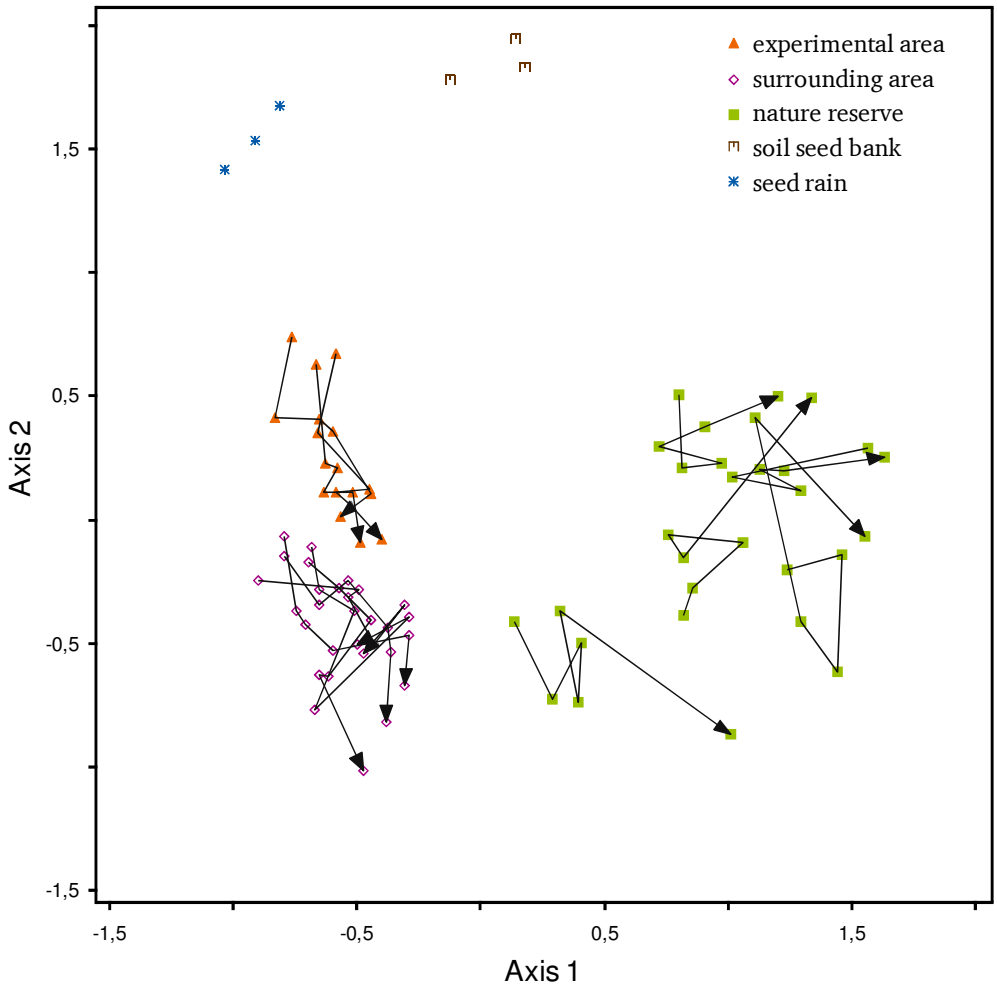


Fig. 3.2: NMDS of presence-absence data of the vegetation on the study sites (2006-2011) and the seed pool samples (2006/07). Time points of single sites are connected by trajectories indicating vegetation development. Final stress: 14.99, final instability: 0.0000, explained variance: axis 1: 52.9 %, axis 2: 32.7 %, orthogonality of axes 100 %, Monte Carlo test $P = 0.001$.

3.5 Discussion

3.5.1 Establishment and persistence of epizoochorously-introduced species

Traditional pastoralism practices have the potential to disperse grassland species and aid in the colonization of restoration sites via epizoochory. In this study, most species dispersed by sheep established on the experimentally-created bare sand areas and persisted during the six-year study. In response to question 1, seeds clearly dispersed and established in relation to sheep grazing. Successful establishment of plant species on bare soil after sheep and goat dispersal was found also by Bugla (2009). This means that sheep can assist grassland restoration by increasing the availability of seeds and encouraging post-dispersal establishment. Information on post-dispersal processes revealed to be as important as information on landscape structure to adequately model landscape connectivity (Rico et al. 2012).

The observed differences in the study species´ establishment rates, recorded as individual numbers, can be explained mainly by differences in life history requirements. Specific requirements of environment determine which species establish successfully in restoration sites. Therefore, future ecological studies should elaborate conditions for the establishment of threatened species. With respect to the first year, differences in detachment rates from sheep fur or in trampling-induced burial-effects (Rotundo & Aguiar 2004; Eichberg et al. 2005) were likely important reasons for different establishment rates of the study species. Additionally, soil chemistry was important; species that successfully established were characteristic of base-rich, nutrient-poor sand, whereas species that are typical for acidic (*J. montana*) or more fertile soils (*T. racemosus*; see Ellenberg et al. 2001) did not perform well.

Another factor influencing the establishment rate and persistence of introduced species is the successional stage of the restoration site. The first years were characterized by low vegetation cover favouring most annual study species. Nevertheless, annual species typically show fluctuating abundances across years (e.g. Špačková & Lepš 2004; Winkler et al. 2011); probably this is caused by variability in environmental conditions. Increasing soil consolidation and total vegetation cover may explain why the density of annual species decreased after 2007/2008 (except for *M. minima*). Annuals often are displaced by perennials during early succession (Alday et al. 2011). Although sheep may facilitate the appearance of annual species by soil disturbance, the experimental grazing of the individual experimental areas with four sheep from 2007-2009 probably did not reach an optimal disturbance

intensity. Prolonging the grazing period of a small flock cannot fully replace short-term impacts of a typical sheep flock comprising some hundred animals.

In contrast, some perennial study species presumably benefited from the initial consolidation of the experimental areas (e.g. *S. canescens* and *S. capillata*). Re-emergence and establishment of *S. canescens*, a threatened mid-successional species, may be explained by seeds incorporated into the soil during the experiment in 2005, that reached favourable conditions for establishment e.g., after disturbance. Soil disturbance usually leads to an activation of the soil seed bank (Milton et al. 1997) and has the potential to enhance seedling establishment (Jentsch et al. 2002b). *Stipa capillata*, a typical later successional species (Allio-Stipetum, Festuco-Brometea), needs longer time spans to establish on restoration sites (Eichberg et al. 2010) than other study species.

3.5.2 Spatial patterns of epizoochorously-introduced species

Several studies have assessed seed-dispersal distances in livestock species (e.g. Fischer et al. 1996; Mouissie et al. 2005b) but very few studies have looked at spatial patterns of establishment following livestock epizoochory. As an answer to question 2, we showed that the epizoochorously-induced spatial patterns persisted throughout our study for most study species. One explanation for this phenomenon could be that dispersal distances of the established plant individuals were short (Cousens et al. 2008).

For woody species, previous studies suggest that seedfall and seedling emergence vary from year to year (Hampe et al. 2008). The spatial similarity between life history stages is often weak for these species (Schupp & Fuentes 1995). This does not correspond to the findings in our study; the initial seed distribution and subsequent plant establishment in the following year was significantly related for the three study species. Secondary dispersal (by animals or abiotic processes; reviewed in Vander Wall et al. 2005) or seed predation may not have been important in our study.

Spatial patterns of most introduced species were aggregated the year after epizoochorous dispersal. Initial patterns of aggregation usually were preserved during the following years. Aggregation and association were weaker in annuals than in perennials reflecting the ephemeral life-history strategy of therophytes. Similarly, the spatial association for adult desert shrubs was stable for at least 20 years or more (Miriti 2007). The clumped distribution we observed in our experiment may have been related to the uneven patterns of sheep

movement across the areas (Wessels-de Wit & Schwabe 2010); uneven patterns of sheep movement were observed in sheep grazing larger areas as well (Rosenthal et al. 2012).

The observed clustering could be beneficial for the colonization on bare sandy soil patches since in harsh environments establishment of some species profits from sheltering effects of vegetation, such as shading (Ryser 1993; Maestre et al. 2003; Padilla & Pugnaire 2006). A better understanding of spatial patterns and their consequences for community development will help to direct habitat management and restoration (Nathan & Muller-Landau 2000; Brooker et al. 2008).

3.5.3 Development of the entire plant community on the experimental areas

In response to question 3, the initial vegetation of the newly created experimental areas was dominated by ruderal species, which has also been true of many other restoration projects (Jongepierová et al. 2007; Rydgren et al. 2010). As stated above, the deep sand used can presumably be excluded as source of seeds for most of these species. Most of the 31 taxa found in our soil samples probably entered the seed bank during the 18-month period after sand deposition. One indication that seeds in the seed bank increased during the study was the high degree of similarity between soil seed bank and standing vegetation of the experimental areas and their surroundings. A further indication is the fact that we found a higher density of seeds in the upper than the lower soil layer. However, at least one species growing on the experimental areas, *Melilotus albus*, likely originated from an extant seed bank, because this species emerged almost exclusively in the sample trays of the lower sand layer and was not found in the vicinity of the study site.

According to our seed-trap study, the dominant source for ruderal species was aerial seed input. In line with other restoration sites carried out in our study region (Stroh et al. 2002; Eichberg et al. 2010), the seed rain comprised to a major extent non-target species. Besides, all target and most other species detected in the seed rain could be related to the standing vegetation on the experimental areas and the nearby surroundings. Similar results were obtained by Auffret & Cousins (2011) on former arable fields and Faust et al. (2012) in a Koelerio-Corynephoretea community.

Many species of grassland ecosystems (including sand species like *Corynephorus canescens*) have short dispersal distances and lack the ability to colonize quickly (see e.g. Jentsch & Beyschlag 2003 for sandy grassland; Erfanzadeh et al. 2010 for salt marshes). Particularly

when there is no source population in the direct vicinity of a restoration site, natural colonization processes via seed rain may be slow (e.g. Verhagen et al. 2001; Buisson et al. 2006). Therefore, differences in species composition and unequal community structures between restoration and target sites persisted after several years (e.g. Pywell et al. 2002; Conrad & Tischew 2011). We found in our experimental areas that species composition differed from nature reserves. Some species characteristic of nature reserves were missing on the experimental areas including *A. alyssoides*, *E. seguieriana*, *O. repens* s.l. and *P. phleoides*.

We conclude that even though sheep grazing in many cases might not be sufficient as an exclusive restoration measure, especially within short time periods, grazing has the potential to establish new populations of threatened plant species in sandy grasslands, e.g. *M. minima* and *S. capillata*.

3.6 Implications for restoration practice

Techniques that create bare-soil patches and implement livestock grazing in restoration areas may be useful to increase species richness of target species. Additional seeds of plant species might be introduced by man-made seed transfer to support a specific community structure. This study suggests that restoration goals to re-establish target species can be aided by livestock grazing if properly managed.

Appendix 3.1: Presence table for all species growing on the experimental areas (n = 3; the number of areas with the species is given) and the nearby nature reserve (n = 5; given is the percentage of plots containing the particular species) in the years 2006 to 2011. Target species are highlighted in red, study species are underlined. Agr = Agropyretea; Art = Artemisietea; compGr = competitive Graminoids; FB = Festuco-Brometea; KC = Koelerio-Corynephoretea; MA = Molinio-Arrhenateretea; oth = other communities; Sm = Stellarietea mediae; w = woody plants.

		Experimental area						Nature reserve					
	Taxa	2006	2007	2008	2009	2010	2011	2006	2007	2008	2009	2010	2011
mean no. of species per experimental area		**49**	**51**	**58**	**59**	**50**	**41**	**32**	**33**	**33**	**34**	**33**	**27**
standard error		**4.6**	**5.4**	**2.6**	**3.0**	**2.0**	**1.2**	**3.7**	**3.9**	**4.0**	**3.6**	**3.7**	**3.2**
KC	*Alyssum montanum* subsp. *gmelinii*	3	3	3	3	3	3	-	-	-	-	-	-
KC	*Phleum arenarium*	3	3	3	3	3	3	-	-	-	-	-	-
FB	*Medicago lupulina*	-	2	1	2	2	-	-	-	-	-	-	-
FB	*Scabiosa canescens*	-	-	-	1	1	1	-	-	-	-	-	-
KC	*Corynephorus canescens*	-	-	-	-	-	-	80	60	60	60	60	40
KC	*Echium vulgare*	-	-	-	-	-	-	20	80	60	60	80	80
KC	*Poa bulbosa*	-	-	-	-	-	-	20	20	20	20	20	20
KC	*Thymus serpyllum*	-	-	-	-	-	-	40	40	40	40	40	60
KC	*Veronica verna*	-	-	-	-	-	-	40	20	20	40	20	20
KC	*Alyssum alyssoides*	-	-	-	-	-	-	60	80	60	60	60	-
KC	*Hieracium pilosella*	-	-	-	-	-	-	20	20	20	-	40	20
KC	*Koeleria glauca*	-	-	-	-	-	-	40	40	40	40	20	-
KC	*Sedum acre*	-	-	-	-	-	-	40	80	100	100	40	-
KC	*Poa badensis*	-	-	-	-	-	-	20	20	20	20	-	-
KC	*Acinos arvensis*	-	-	-	-	-	-	20	20	20	-	-	-
KC	*Herniaria glabra*	-	-	-	-	-	-	-	20	-	-	20	-
KC	*Veronica praecox*	-	-	-	-	-	-	-	-	20	20	-	-
FB	*Artemisia campestris*	-	-	-	-	-	-	20	20	20	20	20	20
FB	*Euphorbia cyparissias*	-	-	-	-	-	-	100	100	100	80	100	100
FB	*Helianthemum nummularium* subsp. *obscurum*	-	-	-	-	-	-	60	60	60	20	40	40
FB	*Ononis repens* s.l.	-	-	-	-	-	-	100	100	100	100	100	100
FB	*Phleum phleoides*	-	-	-	-	-	-	60	60	60	60	60	40
FB	*Salvia pratensis*	-	-	-	-	-	-	20	20	40	20	20	40
FB	*Asperula cynanchica*	-	-	-	-	-	-	20	20	20	-	-	20
FB	*Galium verum* agg.	-	-	-	-	-	-	-	-	20	20	-	-
FB	*Euphorbia seguieriana*	-	-	-	-	-	-	20	-	-	-	-	-
FB	*Linum perenne*	-	-	-	-	-	-	-	-	-	-	20	-
KC	*Arenaria serpyllifolia* agg.	3	3	3	3	3	3	100	100	80	100	100	20
KC	*Armeria maritima* subsp. *elongata*	3	3	3	3	3	3	20	20	20	20	20	20
KC	*Cerastium semidecandrum*	3	3	3	3	3	3	40	40	80	100	80	60
KC	*Medicago minima*	3	3	3	3	3	3	20	60	60	80	60	20
KC	*Rumex acetosella* s.l.	1	1	1	1	1	1	20	20	20	20	20	20
KC	*Potentilla argentea* agg.	-	1	2	1	2	2	60	80	60	40	60	60
KC	*Silene conica*	3	3	3	3	3	3	60	40	20	40	20	-

Appendix 3.1: continued

		Experimental area						Nature reserve					
	Taxa	2006	2007	2008	2009	2010	2011	2006	2007	2008	2009	2010	2011
KC	*Trifolium arvense*	1	2	3	3	2	3	20	40	-	20	20	-
KC	*Helichrysum arenarium*	-	-	2	1	2	2	100	60	40	40	40	-
KC	*Trifolium campestre*	-	-	1	1	3	1	20	40	20	20	20	-
KC	*Vicia lathyroides*	-	2	1	2	2	2	20	-	-	60	20	20
KC	*Erodium cicutarium*	-	-	1	1	-	-	60	100	60	100	100	100
KC	*Myosotis ramosissima*	-	-	3	3	1	1	40	-	-	40	20	20
KC	*Myosotis stricta*	3	3	3	3	2	-	40	-	40	20	-	-
KC	*Petrorhagia prolifera*	1	3	3	3	3	3	40	20	-	-	-	-
KC	*Vulpia myuros*	3	3	3	3	3	3	20	-	-	-	40	-
KC	*Erophila verna*	3	-	3	3	-	-	20	-	-	20	-	-
KC	*Saxifraga tridactylites*	-	-	3	3	2	-	-	-	20	-	-	-
FB	*Koeleria macrantha*	1	1	2	2	2	2	60	40	20	40	40	40
FB	*Stipa capillata*	3	3	3	3	3	3	100	100	100	100	100	100
FB	*Centaurea stoebe* s.l.	3	3	3	3	3	3	80	100	80	60	40	-
FB	*Medicago falcata* (x *varia*)	-	-	-	1	1	1	60	60	60	60	80	60
FB	*Silene otites*	3	3	3	3	3	3	20	20	-	-	-	-
Agr	*Convolvulus arvensis*	3	2	3	3	2	1	-	-	-	-	-	-
Agr	*Carex praecox*	1	-	1	1	1	1	-	-	-	-	-	-
Art	*Artemisia vulgaris*	2	2	2	2	2	1	-	-	-	-	-	-
Art	*Melilotus albus*	2	2	2	2	3	2	-	-	-	-	-	-
Art	*Daucus carota*	1	2	1	1	1	-	-	-	-	-	-	-
Art	*Senecio inaequidens*	-	-	1	3	2	1	-	-	-	-	-	-
Art	*Alliaria petiolata*	1	2	-	-	-	-	-	-	-	-	-	-
Art	*Chelidonium majus*	1	1	-	-	-	-	-	-	-	-	-	-
Art	*Galium aparine*	1	1	-	-	-	-	-	-	-	-	-	-
Art	*Tragopogon dubius*	-	-	1	-	-	1	-	-	-	-	-	-
Art	*Urtica dioica* s.l.	-	-	1	1	-	-	-	-	-	-	-	-
compGr	*Poa compressa*	3	2	2	1	1	1	-	-	-	-	-	-
MA	*Holcus lanatus*	3	3	3	3	3	3	-	-	-	-	-	-
MA	*Trifolium repens*	-	-	1	1	1	1	-	-	-	-	-	-
MA	*Dactylis glomerata*	-	1	1	2	-	-	-	-	-	-	-	-
MA	*Phleum pratense*	-	1	1	1	-	-	-	-	-	-	-	-
MA	*Cerastium holosteoides*	-	-	3	3	-	-	-	-	-	-	-	-
oth	*Securigera varia*	1	1	1	1	1	1	-	-	-	-	-	-
oth	*Tragus racemosus*	3	2	2	2	1	-	-	-	-	-	-	-
oth	*Falcaria vulgaris*	1	1	-	-	-	-	-	-	-	-	-	-
oth	*Parthenocissus inserta*	-	1	-	1	-	-	-	-	-	-	-	-
oth	*Anthemis arvensis*	1	-	-	-	-	-	-	-	-	-	-	-
oth	*Poa annua*	-	2	-	-	-	-	-	-	-	-	-	-
oth	*Portulaca oleracea*	-	1	-	-	-	-	-	-	-	-	-	-

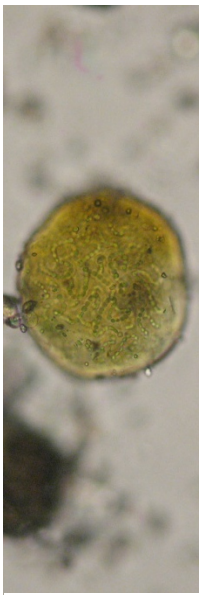
Appendix 3.1: continued

		Experimental area						Nature reserve					
	Taxa	2006	2007	2008	2009	2010	2011	2006	2007	2008	2009	2010	2011
Sm	*Digitaria sanguinalis*	3	2	3	3	-	-	-	-	-	-	-	-
Sm	*Hordeum murinum*	-	1	1	1	-	-	-	-	-	-	-	-
Sm	*Papaver dubium* s.l.	2	1	1	-	-	-	-	-	-	-	-	-
Sm	*Sonchus asper*	3	2	1	-	-	-	-	-	-	-	-	-
Sm	*Alopecurus myosuroides*	-	2	-	1	-	-	-	-	-	-	-	-
Sm	*Capsella bursa-pastoris*	1	-	1	-	-	-	-	-	-	-	-	-
Sm	*Chenopodium pumilio*	2	1	-	-	-	-	-	-	-	-	-	-
Sm	*Lactuca serriola*	1	-	-	-	1	-	-	-	-	-	-	-
Sm	*Bromus sterilis*	-	-	-	-	-	2	-	-	-	-	-	-
Sm	*Cardamine hirsuta*	-	-	1	-	-	-	-	-	-	-	-	-
Sm	*Eragrostis minor*	3	-	-	-	-	-	-	-	-	-	-	-
Sm	*Lamium purpureum*	-	-	1	-	-	-	-	-	-	-	-	-
Sm	*Polygonum aviculare* agg.	1	-	-	-	-	-	-	-	-	-	-	-
Sm	*Senecio vernalis*	-	-	-	1	-	-	-	-	-	-	-	-
w	*Prunus serotina*	3	3	2	2	1	1	-	-	-	-	-	-
w	*Cornus sanguinea*	2	-	2	2	2	1	-	-	-	-	-	-
w	*Robinia pseudacacia*	-	-	1	1	1	1	-	-	-	-	-	-
w	*Pinus sylvestris*	-	1	1	-	-	-	-	-	-	-	-	-
w	*Crataegus monogyna*	-	-	-	2	-	-	-	-	-	-	-	-
Agr	*Cerastium arvense*	-	-	-	-	-	-	20	40	60	40	40	40
Art	*Malva alcea*	-	-	-	-	-	-	20	20	20	20	20	60
Art	*Silene latifolia* subsp. *alba*	-	-	-	-	-	-	20	20	-	-	20	20
compGr	*Carex hirta*	-	-	-	-	-	-	60	60	80	80	80	80
compGr	*Elymus athericus*	-	-	-	-	-	-	20	-	20	-	-	-
MA	*Helictotrichon pubescens*	-	-	-	-	-	-	20	-	40	20	60	20
oth	*Senecio jacobaea*	-	-	-	-	-	-	-	-	20	20	20	-
oth	*Arabis glabra*	-	-	-	-	-	-	-	-	-	20	-	20
oth	*Campanula rapunculus*	-	-	-	-	-	-	20	20	-	-	-	-
Sm	*Psyllium arenarium*	-	-	-	-	-	-	40	60	60	60	80	60
Sm	*Crepis tectorum*	-	-	-	-	-	-	20	20	-	20	20	20
Sm	*Fallopia convolvulus*	-	-	-	-	-	-	-	-	20	20	20	20
Sm	*Corispermum leptopterum*	-	-	-	-	-	-	20	-	20	-	-	-
w	*Ulmus minor*	-	-	-	-	-	-	-	20	20	20	20	20
w	*Rosa* spec.	-	-	-	-	-	-	-	-	-	20	20	-
w	*Rubus caesius*	-	-	-	-	-	-	-	20	20	-	-	-
Agr	*Diplotaxis tenuifolia*	2	1	1	1	-	-	20	20	40	-	-	20
Agr	*Saponaria officinalis*	-	1	-	-	-	-	40	40	40	40	40	40
Art	*Berteroa incana*	1	1	1	1	2	1	20	40	40	40	40	20

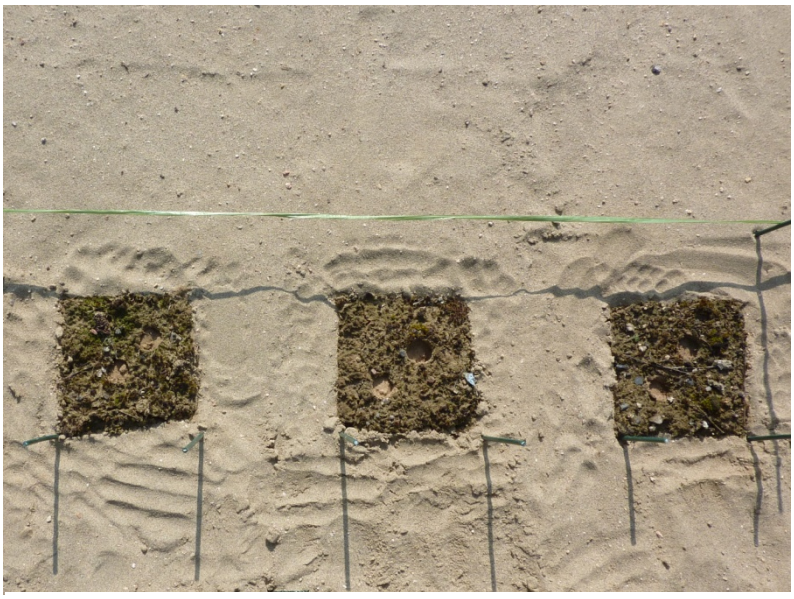
Appendix 3.1: continued

		Experimental area						Nature reserve					
	Taxa	2006	2007	2008	2009	2010	2011	2006	2007	2008	2009	2010	2011
Art	*Cynoglossum officinale*	3	3	3	3	3	3	20	20	40	20	60	60
Art	*Oenothera biennis* agg.	3	3	3	3	3	3	80	40	80	80	80	80
Art	*Carduus nutans*	-	-	1	1	-	-	-	40	-	20	20	40
compGr	*Poa angustifolia/pratensis* agg.	3	3	3	3	3	3	80	80	100	100	100	100
compGr	*Calamagrostis epigejos*	-	1	3	3	3	3	20	40	40	40	40	20
compGr	*Elymus repens*	2	2	2	1	1	1	20	20	20	20	-	-
compGr	*Agrostis capillaris*	-	-	2	1	-	-	-	-	-	-	20	-
MA	*Achillea millefolium*	3	2	3	3	3	3	40	20	40	40	40	20
MA	*Galium album*	3	2	2	3	3	3	20	20	20	20	20	20
MA	*Plantago lanceolata*	3	1	3	3	2	3	20	20	20	20	20	20
MA	*Crepis capillaris*	-	3	3	3	3	3	40	40	80	60	40	20
MA	*Hypochaeris radicata*	-	3	3	3	3	3	-	60	20	-	20	20
MA	*Arrhenatherum elatius*	3	3	3	3	3	3	20	-	-	-	-	-
MA	*Taraxacum officinale* s.l.	-	3	-	-	-	-	-	20	20	-	-	20
oth	*Festuca ovina* agg.	1	1	2	2	2	2	80	80	80	60	60	60
oth	*Rumex thyrsiflorus*	1	3	3	3	3	3	20	40	20	40	40	20
oth	*Verbascum phlomoides*	3	3	3	3	3	2	80	80	100	80	100	100
oth	*Hypericum perforatum*	-	-	1	2	2	2	40	40	40	40	60	60
oth	*Agrimonia procera*	1	1	-	-	-	-	40	20	20	40	20	40
oth	*Geranium molle*	-	-	-	1	-	-	60	60	80	60	40	60
oth	*Asparagus officinalis*	-	-	2	2	1	-	-	20	40	20	-	-
oth	*Vicia angustifolia*	-	-	-	-	2	2	20	20	-	20	-	-
Sm	*Conyza canadensis*	3	3	3	3	3	3	80	80	80	80	100	80
Sm	*Setaria viridis*	3	1	3	2	1	-	40	20	20	60	40	80
Sm	*Bromus hordeaceus*	2	2	-	2	1	2	40	20	20	20	20	-
Sm	*Chenopodium album* agg.	3	3	3	1	1	-	-	20	20	40	60	80
Sm	*Sisymbrium altissimum*	3	3	3	3	3	1	40	40	-	60	60	-
Sm	*Bromus tectorum*	2	3	3	3	3	3	40	20	-	20	-	-
Sm	*Veronica arvensis*	-	-	3	3	2	-	40	40	60	60	40	20
Sm	*Salsola kali* subsp. *tragus*	1	-	-	-	-	-	40	60	40	20	60	40
Sm	*Vicia villosa* s.l.	2	3	1	2	2	1	-	20	-	-	-	-
Sm	*Apera spica-venti*	3	3	3	3	3	-	-	-	-	-	20	-
Sm	*Amaranthus retroflexus*	2	2	-	-	-	-	-	40	-	-	-	20
Sm	*Arabidopsis thaliana*	-	-	2	1	1	-	-	-	-	20	-	-
Sm	*Solanum nigrum*	3	2	-	-	-	-	-	-	-	-	20	60
Sm	*Vicia hirsuta*	-	-	-	-	2	1	-	-	-	20	-	-

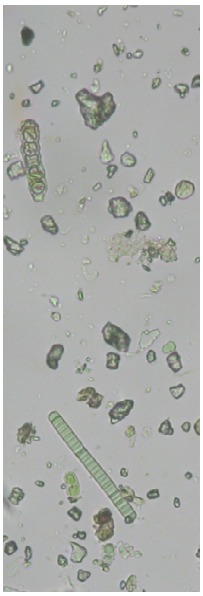
4 Primary succession of biological soil crusts is only slightly affected by crust transplantation and effects are strongly taxa-dependent



Nostoc minutum



transplanted stable biological soil crusts on restoration site 1 ´Apfelbachdüne´, 2011



microscope image

4.1 Abstract

Biological soil crusts (BSCs) are often the first colonizers of undeveloped soil. They show – among others – beneficial effects for soil stabilization and considerable biodiversity in the first successional stages, and should also be taken into account in restoration practice. We investigated the initial development of soil crusts on a newly created restoration site with nutrient-poor bare sand in Central Europe. To study whether the introduction of stable biological crusts has an effect on the establishment and composition of soil crust organisms, 16 soil crust squares (11 cm x 11 cm) derived from a donor site were transplanted onto the restoration site. Whether effects were dependent on distance and time, the community composition of these stable crusts was compared with the composition determined at three distances (5 cm, 50 cm and 200 cm) from the transplants and in control plots over a period of three years. In the same period chlorophyll *a* measurements were conducted for control and the three distances.

Determination revealed significant differences in species and taxa numbers between the transplanted BSCs and control/distances at the date of transplantation. Already one year later community composition had converged on all plots in direction of the transplants as illustrated by ordination and Sørensen indices. Nevertheless, significant differences according to species numbers, total and cyanobacterial taxa numbers remained between transplants and control/distances till the end of the study, whereas differences in taxa numbers of Chlorophyta were dependent on year. Distance- and time-dependent effects of the transplants were determined for total and cyanobacterial taxa numbers as well as for transplanted bryophytes. Among the bryophytes, three out of four species introduced by the transplants were able to establish permanently on the restoration site. Determination of the chlorophyll *a* content of control and the distances revealed an increase from the first to the second year and a slight decline in the third year.

We can conclude that the introduction of stable soil crusts does not greatly affect the establishment and community composition of cyanobacteria and eukaryotic algae during primary succession; the colonization by these organisms is conducted most likely via air. For bryophytes, the introduction of soil crust pieces can have the function of founder populations on the new site.

4.2 Introduction

In many arid and semi-arid ecosystems throughout the world the first millimetres of the top soil are inhabited by a complex community of microorganisms. These biological soil crusts (BSCs) are formed by cyanobacteria, algae, bryophytes, lichens and microfungi aggregating with soil particles (Belnap et al. 2001a). Even though the main occurrence of BSCs is in hot, cool and cold semi-arid and arid areas, they also occur in temperate regions when edaphically dry conditions limit the development of a dense vegetation of vascular plants, which otherwise would outcompete this microorganism community mainly by light limitation (Belnap et al. 2001a). In Central Europe, BSCs occasionally occur in open xerothermic grassland (Büdel 2001a), e.g. in both base-rich (Langhans et al. 2009a) and acidic inland sand dunes (Fischer et al. 2010a), and can build up stable communities persisting more than 20 years (Langhans et al. 2009a).

Biological soil crusts are important components of dry ecosystems and fulfil a variety of ecological functions. In initial soil formation BSCs play an important role by improving the physico-chemical properties of the soil. They stabilize soil surfaces, e.g. through extracellular polysaccharides produced by cyanobacteria (Mager & Thomas 2011), and thus reduce wind and water erosion (Warren 2001; Eldridge & Leys 2003). Additionally, BSCs enhance nutrient availability by fixing nitrogen (mainly by cyanobacteria) and act as carbon sink (Elbert et al. 2012). BSCs have impacts on hydrological processes (Belnap 2006) and, not least, they interact with vascular plants in several ways (Belnap et al. 2001b; Langhans et al. 2009b).

During primary succession, cyanobacteria are often described as the first colonizers, e.g. in the forefield of high-elevation glaciers (Schmidt et al. 2008) or in inland sand dunes (Pluis 1994). According to Belnap & Eldridge (2001) the successional sequence starts with large filamentous cyanobacteria, followed by small cyanobacteria and eukaryotic algae; bryophytes and lichens mostly colonize stabilized soils. However, dominance of a certain taxonomic group depends on, amongst other things, soil properties and moisture. In arid and hyperarid deserts, BSCs are mainly composed of cyanobacteria, whereas in drylands with more humid climate bryophytes and lichens dominate (Belnap 2006). Eukaryotic algae are favoured as well by more humid conditions and on more acidic soils (Belnap et al. 2001a).

Studies on primary succession are, under natural conditions, limited to landscapes completely destroyed or newly created, e.g. by volcanic activities, in forefields of receding glaciers or in coastal and inland sand dune systems (Schaaf et al. 2011). Another area of study for primary succession includes human-disturbed sites like mined areas (Lukešová 2001; Song et al.

2014), though these sites may take altered successional pathways due to special soil conditions or contaminations with toxic elements of the spoil. There is one study in Central Europe which uses an artificially created sandy site to record the initial ecosystem development (Fischer et al. 2010b).

Introduction of soil-crust organisms was employed in several studies on restoration of BSCs to speed up the recreation of crust cover in disturbed areas. Thereby, the crust organisms were introduced, e.g., via laboratory-prepared inoculation material (Wang et al. 2009), in a slurry prepared from soil crusts (Maestre et al. 2006) or via crumbled crusts of nearby areas (Belnap 1993). Studies dealing with the transplantation of crust pieces are rare and those we have found are focussed on bryophytes and lichens. Bowler (1999) observed survival and expansion of transplanted bryophytes and liverworts in several plots, whereas the crust-transplanted bryophyte described by Cole et al. (2010) survived indeed, but declined in cover. Scarlett (1994) only stated that ´piece placing will transfer all species successfully if soil type and micro-environments are matched´, but it does not become clear which specific species/taxonomic groups were investigated. Lateral spread was not studied by Cole et al. (2010) and Scarlett (1994).

Here, we present a study on the establishment of a soil crust on a site that was newly created by deposition of deep sand in the course of a restoration project. The target vegetation for this site is the highly endangered Koelerion glaucae vegetation complex, which is characterized also by BSCs as documented by Hach et al. (2005) and Langhans et al. (2009a) for our area. The vegetation development after inoculation with Koelerion glaucae plant material on this restoration site is shown by Freund et al. (Tuexenia; see Chapter 2).

In the present study small squares of stable BSCs (> 20 years old) from a nearby nature protection area were transplanted onto this restoration site. The development of the transplanted BSCs and of the initial soil crust at three different distances from the transplants as well as of control plots was monitored over a period of three years, both via determination and via chlorophyll *a* measurement. The following questions were addressed:

(1) Which species/taxa occur on a newly created restoration site after one and two years of crust development, respectively?

(2) Does the introduction of stable biological soil crust transplants have an impact on species composition of the newly developing crusts? Is this impact dependent on distance and time? Are the analysed taxonomic groups (cyanobacteria, eukaryotic algae, bryophytes) affected differently?

(3) Is the development of BSCs reflected by the chlorophyll *a* content?

4.3 Methods

4.3.1 Study site

The study site (restoration site 1, ´Apfelbachdüne´; 8°35' E, 49°56' N; see Chapter 2) is situated in the northern Upper Rhine Valley, Germany, about 20 km south of Frankfurt/Main. On a formerly arable field nutrient-poor deep sand (> 1.5 m depth) was deposited in summer 2009 to obtain a restoration site with low nutrient status (size: approx. 1.1 ha). The deep sand for the study site was obtained from a construction site.

Soil analysis of the experimental areas (see below) revealed mean values of pH 7.56 ± 0.01, phosphate-P = 7.27 ± 0.26 mg kg^{-1}, nitrate-N = 0.15 ± 0.04 mg kg^{-1} and ammonium-N = 0.15 ± 0.02 mg kg^{-1} (± SE; n = 4; Lingen 2013). Samples for soil analysis were taken in Oct. 2011 from a depth of 0-10 cm; analyses were conducted as described by Storm & Süss (2008). A soil expert's examination of the sand revealed the assignment criterion Z0 according to LAGA-M20, i.e. the sand is environmentally compatible.

The mean temperature was 10.6 °C, annual precipitation amounted to 612 mm and the annual duration of sunshine was 1678 h (1984-2013, Frankfurt/Main airport, Deutscher Wetterdienst; www.dwd.de). The climatic conditions in March differed between the last sampling year (2013) and the previous ones; mean temperature and duration of sunshine were lower (2.8 °C, 124 h) compared to 2011 (7.6 °C, 213 h) and 2012 (9.0 °C, 178 h), precipitation was higher (27 mm) than in 2011 (16 mm) and 2012 (15 mm).

4.3.2 Study and sampling design

In total, four experimental areas were installed on the restoration site in a systematic arrangement. Each experimental area was 70 m^2 in extent with a control plot in the western and a sampling plot in the eastern part, each with 5 m^2 (Fig. 4.1 a). The experimental areas were fenced against grazing by donkeys; additionally, control and sampling plots were fenced with chicken fence (50 cm high and 20 cm below-ground) against hares and other small mammals. On both control and sampling plots the topmost 1-2 cm of sand were removed to eliminate the already established initial soil crust and to mimic the bare substrate right after modelling of the restoration site. On the downward slope part of each sampling plot four squares of intact BSCs (11 cm x 11 cm), obtained from a donor site, were placed in March 2011 (Fig. 4.1 b). The BSC-squares were separated by distance of 11 cm from the plot sides and between each other.

Per transplanted BSC-square (T) two samples of 1.9 cm diameter (2.84 cm^2) and 1 cm depth were taken after moistening with distilled water, i.e. eight samples per plot; sampling of the intact BSCs followed the diagram depicted in Fig. 4.1 d. Additionally, the developing soil crust on the bare soil was sampled on the control plot (C; at least 6 m from T) and at distances of 5 cm, 50 cm and 200 cm from the intact BSC-squares. Per distance and per control plot, eight samples (1.9 cm diameter, ca. 1 cm deep) were taken at intervals of 9 cm (Fig. 4.1 c). Additional samples for chlorophyll *a* analyses were taken directly above those for determination from control plot and the three distances. The first sampling took place in March 2011 on the day of transplantation, followed by two samplings 12 and 24 months later. The samples for determination were dried at 35 °C for about 48 h before storage under dark and dry conditions until determination; the samples for chlorophyll *a* analyses were stored at -18 °C until extraction.

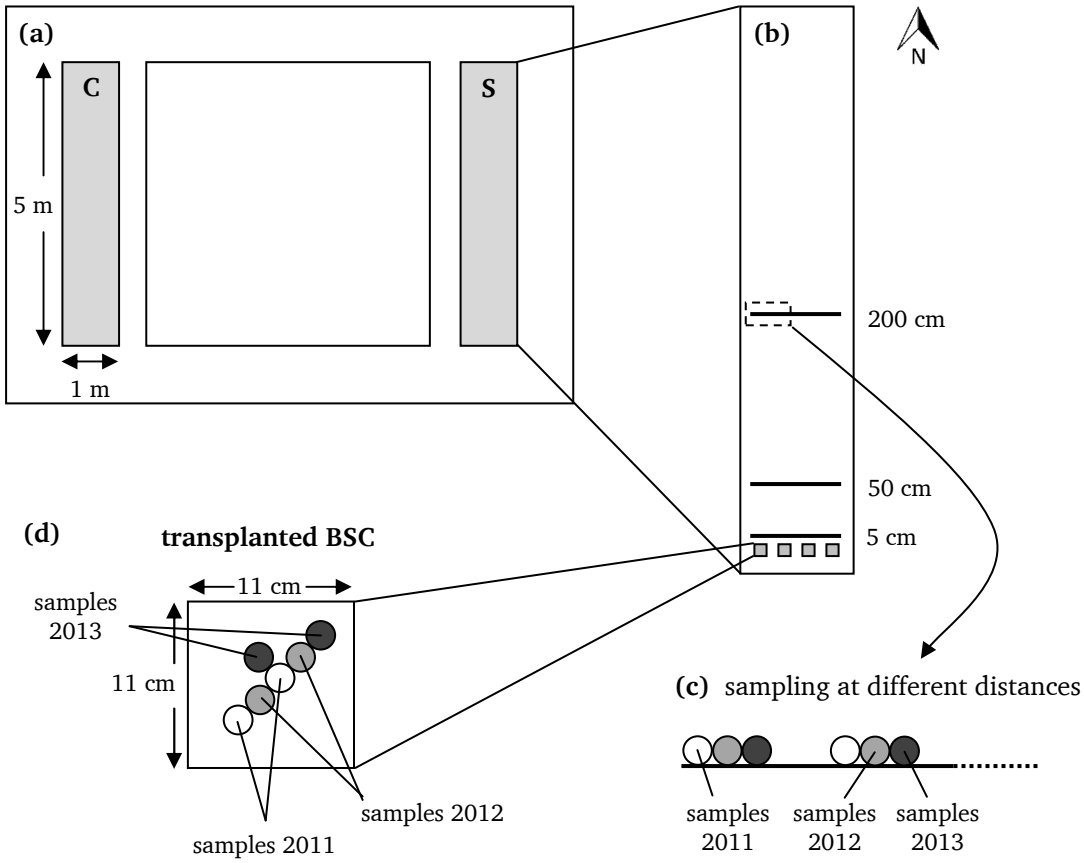


Fig. 4.1: Diagram of the experimental design. (a) Fenced experimental area with control plot (C) and sampling plot (S). (b) Detailed design of the sampling plot with transplanted BSCs (gray squares) and the three sampling distances. Sampling design of (c) the control, the different distances from the transplanted BSCs and (d) of the transplanted BSCs in 2011-13.

4.3.3 Donor site

As donor site for the intact BSCs we used a nature protection area (´Ehemaliger August-Euler-Flugplatz von Darmstadt´, 8°35' E, 49°51' N). The eastern part of this area consists of pioneer plant communities of the Koelerion glaucae vegetation complex, providing an open vegetation structure allowing the occurrence of BSCs (Hach et al. 2005).

The intact BSCs (stable crusts of at least 20 years age) were taken from a plot fenced against rabbits and free ranging livestock. Sixteen crust-squares were obtained, four on each of four locations within this plot. To obtain the BSCs, aluminium frames (11 cm x 11 cm) were pushed about 2 cm deep into the soil, the crusts were then cut out by metal plates and fixed in the frames for transportation to the restoration site. The proportion of bryophytes on total cover of the sampled BSCs was less than 5 (10) %. Lichens were not observed in the BSCs. Phanerogams were absent in the crusts except for one seedling.

4.3.4 Determination

Before determination the crusts were rewetted for at least one hour. For determination a Zeiss Axioskop 40 was used. Identification of the organisms was conducted according to Geitler (1932), Ettl & Gärtner (1995), Komárek & Anangnostidis (1999) and Komárek & Anangnostidis (2005). Bryophytes were determined according to Frahm & Frey (2004) and lichens according to Wirth et al. (2013).

Additionally, percentage cover data of bryophytes in the crust samples were assessed, using the following scale: 0.1, 1, 2,... 6, 8, 10, 12, 15, 20,... 100 %. Estimation of crust cover was not possible due to the initial stages of the BSCs.

4.3.5 Chlorophyll *a* determination

Prior to extraction, macroscopic visible parts of bryophytes (except for their protonemata) were removed from the thawed samples. Chlorophyll *a* was extracted in dimethyl sulfoxide (DMSO) according to the method of Ronen & Galun (1984). Approximately 1 g of the slightly crushed soil substrate was mixed with 25 mg $CaCO_3$ (to avoid acidification and the resulting pheophytinization of chlorophyll) and 4 ml DMSO (99.5 %) and heated for 90 min at 65° C in a water bath. The supernatant was decanted (extract 1) and stored in the dark. Another 4 ml DMSO were added to the sample and heated as described above. The supernatant was again

decanted, merged with extract 1 and centrifuged for 5 min (4000 rpm). The chlorophyll content was calculated according to Arnon (1949) after spectrophotometry with a Spectronic Genesys 5 (2011, 2012) and a WPA Biowave DNA (2013).

4.3.6 Data analysis

The composition of taxa in the BSCs was analysed by means of detrended correspondence analysis (DCA) using PC-Ord 6.17. Analysis was run on frequency values, which were calculated from the presence/absence data for each treatment/distance and year per plot. The DCA was run with downweighting of rare species and rescaling; the number of segments was 26.

Similarity of community composition between C and T and between both and the plots at the three distances in each of the years was expressed with the Sørensen index.

The impact of ´treatment/distance´ and ´year´ on numbers of species (only organisms determined to species level) and taxa (all observed organisms) were analysed by means of mixed linear models (SAS 9.2, PROC GLIMMIX; SAS Institute Inc., Cary, NC, USA; Littell et al. 2006). Especially for analysis of repeated-measures data the use of mixed linear models is suitable as they allow comparison of the goodness of fit of several covariance structures (Littell et al. 1998). Fourteen covariance structures were tested, whereof the structure with the best goodness of fit according to the corrected Akaike criterion (AICC; Fernández 2007) was chosen for final calculations. When two covariance structures resulted in equal AICC values, the simpler one was chosen. For calculating the degrees of freedom, the Kenward-Roger approximation was selected (Schaalje et al. 2002). Using this method, mixed linear models are robust against deviation from normal distributions in terms of both error control and power (Vallejo et al. 2004; Jacqmin-Gadda et al. 2007). Nevertheless, the studentized residuals and conditional studentized residuals were examined for normality by means of graphical display (histograms and quantile-residuum plots); a nearly Gaussian distribution could be ascertained. Tukey-adjusted post hoc tests were carried out to test for effects of treatments/distances within each year.

To compare all treatments/distances with the control, Dunnett-adjusted post-hoc tests were performed for each separate year using the LSMEANS procedure of PROC GLIMMIX. Additionally, the ESTIMATE procedure was used for analysis of differences between the transplant and the other treatments/distances.

4.4 Results

4.4.1 Determination

During the whole study period a total of 21 species (39 taxa) were detected on the restoration site including eight species (19 taxa) of cyanobacteria, six species (13 taxa) of eukaryotic algae, six species (six taxa + the accumulative group of ´other acrocarpous mosses´) of bryophytes and one lichen (Table 4.1). Treatment/distance and year as well as the combination of both had significant effects on the occurrence of species and taxa (Table 4.2).

In 2011, species/taxa were almost exclusively found in the transplanted BSCs; control and the three distances had significantly lower species/taxa numbers (Table 4.1, Fig. 4.2 a and b). Concurrently, control and distances did not differ significantly (Table 4.3). Even though species and taxa numbers increased in control and the distances in 2012 and 2013, the numbers remained significantly lower than in the transplants. In 2013 the species numbers of the transplants were still 1.4- to 1.6-fold higher than in the 5 cm-distance and the control, respectively; the taxa numbers were 1.3-fold (5 cm) to 1.6-fold (control) higher in the transplant. Simultaneously, the control had significantly lower species numbers than the distances in 2012; in 2013 this difference was no more significant. According to the total taxa numbers, the dissimilarity between control and the 5 cm- and 50 cm-distance increased; the numbers were significantly higher in the 5 cm-distance (2012, 2013) and the 50 cm-distance (2013) than in the control.

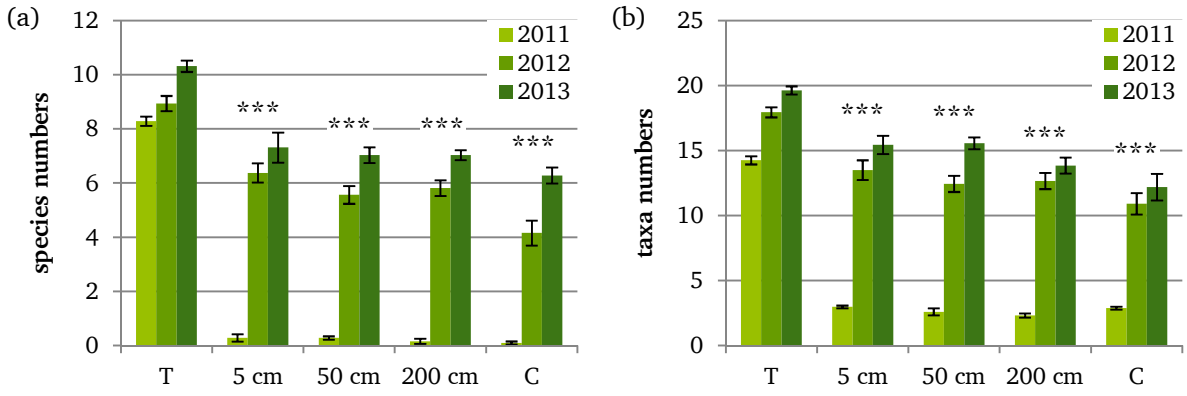


Fig. 4.2: (a) Mean species numbers and (b) mean of total taxa numbers (± SE; n = 4). T = transplanted BSC, C = control. Treatment/distance effects that are significantly different from the transplants are marked with asterisks.

Table 4.1 (next page): Presence table of the species in the three studied years (in %; n = 32). T = transplanted BSC, C = control, 5 – 200 = distance [cm] to the transplants.

Year	2011					2012					2013				
Treatment/Distance to transplant [cm]	T	5	50	200	C	T	5	50	200	C	T	5	50	200	C
Cyanobacteria															
Chroococcus pallidus	88	6	3	-	-	91	59	31	47	25	97	97	66	84	59
Gloeocapsa compacta	97	-	-	-	-	78	6	-	-	13	84	28	16	-	-
Lyngbya spec.	78	3	9	-	-	81	78	75	78	63	94	69	69	88	66
Microcoleus cf. *paludosus*	3	-	-	-	-	-	-	-	-	-	-	6	-	3	-
Microcoleus vaginatus	94	-	-	-	-	100	91	63	75	56	75	63	63	72	63
Nostoc commune	81	-	-	-	-	88	81	88	78	41	100	100	88	100	91
Nostoc cf. *microscopicum*	63	-	-	-	-	72	69	63	50	16	72	53	63	44	66
Nostoc minutum	34	-	-	-	-	31	-	-	3	-	69	6	13	19	3
Nostoc spec.	88	-	-	-	-	88	-	22	9	-	97	66	66	9	6
Oscillatoria spec. 1	100	13	9	9	6	97	91	100	94	81	97	97	97	84	78
Oscillatoria spec. 2	28	-	-	-	-	47	59	59	59	56	41	63	66	53	56
Oscillatoria spec. 3	28	-	-	-	-	53	28	28	31	16	66	38	53	38	6
Oscillatoria spec. 4	63	-	-	-	-	56	31	13	9	25	19	3	13	6	38
Oscillatoria spec. 5	31	-	-	-	-	19	9	13	3	3	53	28	22	-	9
Phormidium spec.	59	3	-	-	6	34	47	28	41	47	19	44	47	16	31
Synechococcus cf. *sciophilus*	47	-	9	3	-	31	16	3	3	6	6	9	3	-	-
indet. Oscillatoriales 1	9	-	-	-	-	22	19	9	25	16	13	6	3	-	6
indet. Oscillatoriales 2	28	-	-	-	-	3	13	-	-	6	31	25	25	-	13
indet. Oscillatoriales 3	6	-	-	-	-	3	-	-	-	-	-	-	-	-	-
Eukaryotic Taxa															
Chlorophyta															
Chlamydomonas spec.	3	-	-	-	-	31	9	6	9	22	41	38	41	47	6
Chloridella/Pleurochloris spec.	50	6	-	9	3	41	44	34	41	41	63	19	38	41	47
Chlorococcum cf. *infusionum*	25	-	6	-	3	13	28	13	22	31	78	59	81	72	25
cf. *Cylindrocystis spec.*	-	-	3	-	3	16	-	-	-	6	16	-	3	3	-
Gloeocystis cf. *vesiculosa*	9	-	3	-	3	22	6	-	-	-	13	3	3	3	-
Klebsormidium cf. *dissectum*	13	-	-	-	-	13	44	28	47	56	-	19	6	-	50
Klebsormidium flaccidum/klebsii	16	3	3	3	-	56	84	88	88	94	88	66	91	100	66
Scenedesmus acutus	28	-	-	-	-	9	-	-	-	-	28	13	16	6	6
Zygogonium ericetorum	100	19	3	9	3	100	75	100	97	69	100	100	97	100	100
Heterokontophyta															
Pennales 1	6	100	88	78	88	91	94	97	97	100	91	100	100	97	72
Pennales 2	3	91	72	63	94	94	94	100	94	97	56	91	84	100	69
Pennales 3	6	53	50	56	78	94	97	97	94	88	91	100	100	100	69
Pennales 4	9	-	-	-	-	31	-	6	-	9	47	28	28	-	19
Bryophytina and Lichenes															
other Acrocarpi	-	-	-	-	-	-	3	9	3	3	6	31	34	16	9
Bryum argenteum	-	-	-	-	-	59	53	81	72	9	94	94	97	100	100
Ceratodon purpureus	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
Tortella inclinata	56	-	-	-	-	69	16	-	-	-	66	3	-	-	-
Tortella tortuosa	3	-	-	-	-	3	3	-	-	-	6	3	-	-	-
Tortula ruraliformis	69	-	-	-	-	59	6	-	-	-	56	6	3	-	-
Hypnum cupressiforme	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sarcogyne regularis	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-

Table 4.2: Results of the linear mixed models for the analysed variables. Significant results are shown in bold print. P = level of significance.

	treat	**year**	**treat*year**
Variable	*P*	*P*	*P*
Species numbers	**<.0001**	**<.0001**	**<.0001**
Taxa numbers			
total	**<.0001**	**<.0001**	**<.0001**
Cyanobacteria	**<.0001**	**<.0001**	**<.0001**
Chlorophyta	**<.0001**	**<.0001**	**<.0001**
Heterokontophyta	**0.0056**	**<.0001**	**<.0001**

Table 4.3: Results of the Dunnett-adjusted post-hoc tests for the analysed variables in separate years. Significant results are shown in bold print. For abbreviations see Fig. 4.2. P = level of significance.

	C-T	**C-5 cm**	**C-50 cm**	**C-200 cm**
Variable	*P*	*P*	*P*	*P*
Species numbers				
2011	**<.0001**	0.6007	0.6007	0.9836
2012	**<.0001**	**0.0015**	**0.0400**	**0.0149**
2013	**<.0001**	0.0624	0.2119	0.2119
Taxa numbers				
total				
2011	**<.0001**	0.9765	0.5210	0.0705
2012	**<.0001**	**0.0146**	0.1765	0.1085
2013	**<.0001**	**0.0122**	**0.0094**	0.2747
Cyanobacteria				
2011	**<.0001**	0.9597	0.8568	1.0000
2012	**<.0001**	**0.0087**	0.1739	0.1237
2013	**<.0001**	**0.0113**	**0.0297**	0.9765
Chlorophyta				
2011	**<.0001**	0.7880	0.9981	0.9748
2012	0.9239	0.7610	0.3213	0.9581
2013	**0.0002**	0.8539	**0.0102**	**0.0134**
Heterokontophyta				
2011	**<.0001**	0.9072	0.1527	0.0585
2012	0.8191	0.9619	0.9910	0.9619
2013	0.6130	0.2397	0.2908	0.4532

Cyanobacteria

Treatment, year and their combination had significant effects on taxa numbers of cyanobacteria (Table 4.2). The transplants maintained significantly higher taxa numbers compared to control and the distances during the study period; in 2013 the values were 1.3- to 1.7-fold higher (5 cm and control, respectively; Fig. 4.3, Table 4.3). In contrast, comparison of control and the distances revealed effects of distance and time on taxa numbers; in 2012 the numbers were significantly higher in the 5 cm-distance than in the control and in 2013 this applied to both in the 5 cm- and 50 cm-distances.

The majority of occurring cyanobacteria were filamentous ones (twelve taxa); both Chroococcales and Nostocales had three taxa each (Table 4.1). The most frequently detected cyanobacteria were *Microcoleus vaginatus* and *Nostoc* cf. *commune*, followed by *Lyngbya* spec. and one *Oscillatoria* which were already detected on the restoration site in 2011.

Compared to eukaryotic algae, taxa numbers of cyanobacteria were 4.2-fold higher in the transplanted BSCs in 2011. In 2012, cyanobacteria had in between 1.5- to 3.3-fold (C and T, respectively) higher taxa numbers than eukaryotic algae. This ratio diminished to 2.4-fold more cyanobacteria taxa in the transplants in 2013; in the control it increased to 2-fold higher taxa numbers of cyanobacteria.

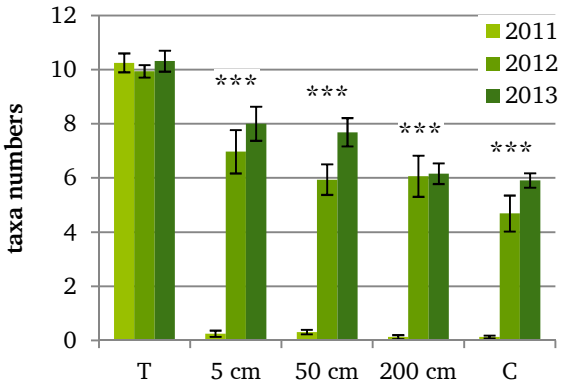


Fig. 4.3: Mean taxa numbers of cyanobacteria (± SE; n = 4). For abbreviations see Fig. 4.2. Treatment/distance effects that are significantly different from the transplants are marked with asterisks.

Eukaryotic algae

On the restoration site six species (nine taxa) of Chlorophyta and four taxa of Heterokontophyta occurred (Table 4.1). For both Chlorophyta and Heterokontophyta significant effects of treatment, year and the combination of both were determined (Table 4.2).

The taxa numbers of Chlorophyta increased in all treatments/distances, whereupon the numbers were higher in the transplants than in control and the distances in 2011 (8.7- to 15.6-fold) and 2013 (1.1- to 1.4-fold); in 2012 the numbers of all treatments/distances ranged around the same value (Fig. 4.4, Table 4.3). In 2013, the 50 cm- and 200 cm-distances revealed significantly higher taxa numbers than the control; the 5 cm-distance had insignificantly higher values. Most frequently detected were the filamentous green algae *Zygogonium ericetorum* and the accumulated group *Klebsormidium flaccidum/klebsii*.

Heterokontophyta were recorded on the restoration site since 2011 with three taxa, another taxon was introduced by the transplants. Thus, the transplants had significantly less taxa (only one) in 2011 than the other treatments/distances (Table 4.3). Subsequently, no significant differences were determined between the treatments and the distances.

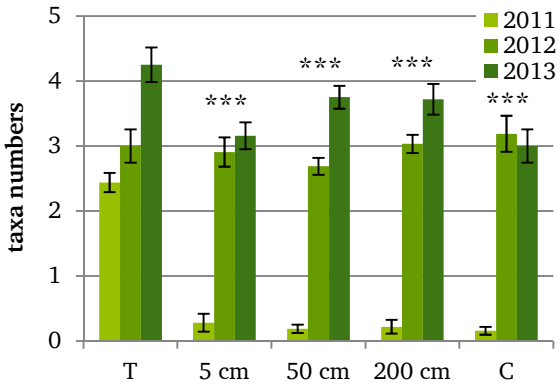


Fig. 4.4: Mean taxa numbers of Chlorophyta (± SE; n = 4). For abbreviations see Fig. 4.2. Treatment/ distance effects that are significantly different from the transplants are marked with asterisks.

Bryophytes and lichens

Of the six bryophyte species detected on the restoration site, four species originated from the transplants including the single pleurocarpous moss *Hypnum cupressiforme* (detected only once in 2011; Table 4.1). The other three ´transplant´-bryophytes showed colonization of the surrounding since 2012, whereby *Tortula ruraliformis* reached the 5 cm-distance (2012) and the 50 cm-distance (2013) and *Tortella inclinata* and *T. tortuosa* only colonized the 5 cm-distance (all with low cover values). In transplants the total bryophyte cover increased during the study period, mainly due to an increase in cover of *T. inclinata* and *Bryum argenteum*.

Independent of the transplants two bryophyte species emerged on the restoration site since 2012, of which only *B. argenteum* was frequently detected. This species was detected in all treatments/distances, also colonizing the transplant, and increased its cover from 2012 to 2013 considerably (Table 4.4).

The lichen *Sarcogyne regularis* was only once detected in a transplant in 2012.

Table 4.4: Total cover and cover of selected bryophytes (in %; mean ± SE; n = 4). For abbreviations see Fig. 4.2.

		2011	2012	2013
total	T	2.80 ± 0.84	5.14 ± 1.99	9.01 ± 3.43
	5 cm	0 ± 0	0.25 ± 0.04	3.64 ± 1.38
	50 cm	0 ± 0	0.46 ± 0.21	5.60 ± 1.45
	200 cm	0 ± 0	0.54 ± 0.23	3.66 ± 1.05
	C	0 ± 0	0.01 ± 0.01	5.75 ± 0.46
Bryum argenteum	T	0 ± 0	0.18 ± 0.09	2.50 ± 1.13
	5 cm	0 ± 0	0.20 ± 0.07	3.19 ± 1.52
	50 cm	0 ± 0	0.46 ± 0.21	5.14 ± 1.51
	200 cm	0 ± 0	0.54 ± 0.23	4.10 ± 0.79
	C	0 ± 0	0.01 ± 0.01	5.75 ± 0.46
Tortula ruraliformis	T	1.96 ± 0.59	1.98 ± 1.04	1.50 ± 0.32
	5 cm	0 ± 0	0.01 ± 0.00	0.03 ± 0.03
	50 cm	0 ± 0	0 ± 0	0.00 ± 0.00
	200 cm	0 ± 0	0 ± 0	0 ± 0
	C	0 ± 0	0 ± 0	0 ± 0
Tortella inclinata	T	0.75 ± 0.29	2.54 ± 0.83	4.61 ± 3.58
	5 cm	0 ± 0	0.02 ± 0.01	0.13 ± 0.13
	50 cm	0 ± 0	0 ± 0	0 ± 0
	200 cm	0 ± 0	0 ± 0	0 ± 0
	C	0 ± 0	0 ± 0	0 ± 0

4.4.2 Similarity in species composition

DCA revealed a separation of the transplants from the sampled distances and the control along the first axis (Fig. 4.5). The different distances and the control show very similar developments; these treatments and the transplants develop towards each other from the first to the second study year. The third study year is separated from the previous ones along the second axis; accordingly, the different distances and the control develop parallel to the transplants. In tendency, the distances of 5 cm and 50 cm are arranged closer to the transplants than the 200 cm-distance and the control (in 2012 and 2013).

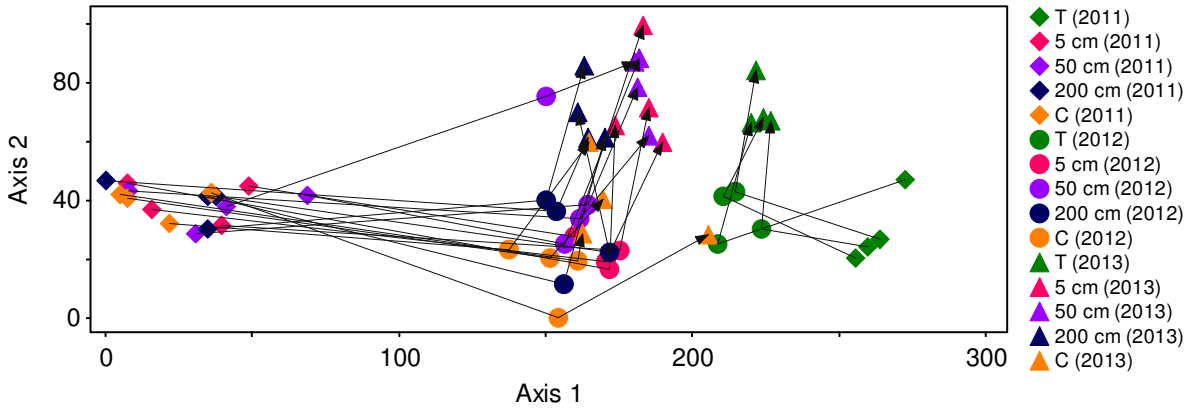


Fig. 4.5: DCA performed with frequency data of the treatments/distances between 2011 and 2013. The time points of each treatment/distance are connected by trajectories. For abbreviations see Fig. 4.2. Eigenvalues: axis 1: 0.22, axis 2: 0.04, axis 3: 0.02.

Like the ordination, the Sørensen indices revealed a convergence of the different distances and the control in direction of the transplants from 2011 to 2013 (Table 4.5). Thereby, the convergence in species/taxa composition was strongest from the first to the second year and was more pronounced in the three distances (especially in 5 cm and 50 cm) than in the control. Concurrently, the dissimilarity between control and 5 cm or 50 cm, respectively, increased slightly from 2011 to 2013.

Table 4.5: Sørensen indices comparing the taxa composition between transplanted BSCs (T), control (C) and with the three distances, respectively, within each year 2011 to 2013.

		5 cm	50 cm	200 cm	C
2011/2011	**T**	0.920	0.926	0.940	0.953
	C	0.155	0.166	0.193	-
2012/2012	**T**	0.218	0.250	0.250	0.341
	C	0.162	0.193	0.161	-
2013/2013	**T**	0.199	0.187	0.234	0.306
	C	0.204	0.195	0.199	-

4.4.3 Chlorophyll *a* content

A significant effect of ´year´ was shown for the chlorophyll *a* content (P <0.0001); it increased considerably from 2011 to 2012 and slightly decreased from 2012 to 2013 (Table 4.6). Despite a high variability between control and distances, ´treatment´ did not affect the chlorophyll content significantly. The variability within the single treatments was high and ranged, e.g., in 2013 between 3.0 mg m^{-2} and 95.6 mg m^{-2} in the 50 cm distance.

Table 4.6: Mean chlorophyll *a* contents (mg m^{-2}; ± SE; n = 4) of control (C) and the three distances from 2011 to 2013.

	2011	2012	2013
C	0.72 ± 0.22	57.22 ± 8.26	31.15 ± 5.32
5 cm	0.59 ± 0.49	44.61 ± 4.35	39.46 ± 8.64
50 cm	0.19 ± 0.04	45.18 ± 3.60	42.15 ± 10.17
200 cm	0.34 ± 0.26	54.02 ± 6.78	38.60 ± 10.88

4.5 Discussion

4.5.1 Occurrence of species

Already after 12 months an initial soil crust had established on the bare sand of all plots including the control. On mining dumps, soil-crust organisms had been found to establish on bare substrate within 3-6 months (Lukešová & Komárek 1987); Dümig et al. (2014) estimated around four years for development of BSCs on an experimental sand dune in Central Europe.

Almost all species forming the initial crust were present in the studied plots after one year; the composition of detected taxa did not change between 12 and 24 months. Only one additional species, the bryophyte *Ceratodon purpureus*, was detected after two years. Lukešová & Komárek (1987) described increasing species numbers until a maximum was reached after 18-19 years in a mining-dump chronosequence. However, it should be considered that species numbers were comparatively high in our study after only one year.

Many species detected in the initial crusts are common soil taxa with broad ecological amplitude; especially the most frequently recorded are found worldwide, including e.g. the cyanobacterial genera *Microcoleus* and *Nostoc* (Büdel 2001b). These genera were found in all sites along a European transect from Sweden to Spain (Büdel et al. 2014) as well as in arid regions from Middle East to southern Africa and Australia (Büdel et al. 2009; Kidron et al. 2010; Williams & Büdel 2012). *Microcoleus* is one of the functionally most important genera in forming crusts (Belnap & Gardner 1993); *Nostoc* is an important nitrogen fixer (Maqubela et al. 2009). Within the frequently detected eukaryotic algae, the genus *Klebsormidium* has a cosmopolitan distribution (e.g. Johansen 1993; Büdel et al. 2009), whereas *Zygogonium ericetorum* is common in European temperate regions (Büdel 2001a). The occurrence in initial (and stable) BSCs in Hessian inland sand dunes was described for all determined species by Langhans et al. (2009a).

As described for various base-rich arid regions (see Belnap & Lange 2001), more cyanobacteria than eukaryotic algae were found in our initial soil crusts, on average at a ratio of 2:1. Crusts on acidic soils were dominated by eukaryotic algae in Central Europe (Lukešová 2001). Nevertheless, the number of eukaryotic algae may be underestimated as we did no culturing (Langhans et al. 2009a); many fragments and reproductive states (presumably of filamentous chlorophyta) were observed, which could not be identified by direct determination. Furthermore, this could be a possible explanation for the lack some species compared to developed biological crust systems in the study area (Langhans et al. 2009a).

4.5.2 Impact of transplanted BSCs

Depicted in the ordination, an approximation of the initial crusts and the transplanted BSCs between 2011 and 2012 becomes apparent. The taxa composition of cyanobacteria and eukaryotic algae in the initial crusts generally corresponded to the composition determined in the transplanted BSCs; however, this applied also for the control. Hence, the impact of the transplants can be considered to be rather low for these organisms. Most likely these cyanobacteria and eukaryotic algae were introduced to the restoration site via wind. Many genera of soil-crust forming algae can be found in air samples (Schlichting 1969; Kristiansen 1996). At the beginning of our experiment in 2011 (around 1.5 years after construction) an initial soil crust had already established on the restoration site, whose development can only be explained by airborne colonization. The sand used for construction of the site was presumably almost free of organisms as it was obtained from a depth greater than 1.5 m. The surrounding initial crust might have enhanced the colonization speed of our plots; re-establishment might have taken place again via air, from the border of the plots or from the subjacent sand (as some species were already detected on the bare sand in 2011).

However, the transplanted BSCs affected the nearby distances at least in two ways according to distance and time. At first, transplants had an effect on total and cyanobacterial taxa numbers; both were enhanced in the vicinity of the transplants compared to the control. The number increased in distances of 5 cm and 50 cm with time since transplant introduction (after one and two years, respectively). According to cyanobacteria, this might be connected with the ability of some species to move actively (e.g. Garcia-Pichel & Pringault 2001).

Secondly, a spread of bryophytes could be observed away from the transplants. At least three bryophytes were introduced to the restoration site by the transplants; of these, two species were able to colonize the 5 cm distance (in 2012 and 2013; *T. inclinata*) and one additionally the 50 cm distance (in 2013; *T. ruraliformis*). Bowler (1999) reported expansion of transplanted bryophytes and liverworts in several plots, but it is not clear whether propagation inside of plots or dispersal to the surrounding is meant. Cover of the bryophytes on the newly colonized distances was rather low, presumably as their cover in the transplanted BSCs was low due to our transplant selection. For lichens, Lalley & Viles (2008) described the overall lichen cover in bordering areas to influence recovery rates most strongly. A decline of bryophyte cover after transplanting bryophyte soil crusts as reported by Cole et al. (2010) in the Mojave Desert seems to be dependent on the particular species and maybe on the studied ecosystem. In our study the cover of *T. ruraliformis* decreased slightly in the

transplants, whereas the cover of *T. inclinata* increased. Another bryophyte species, *B. argenteum*, was shown to colonize nearly all plots since the second sampling date regardless of the transplants. Hence this species may have caused, amongst others, the approximation of the transplants to control and the distances as shown by the ordination.

Concluding that at least in our study system the benefits of transplanting BSC pieces were rather low according to species composition of algal organisms, it should also be taken into account that the removal of crusts has also an impact on the donor system. Even fine-scale disturbance was shown to result in a successional replacement of BSCs by bryophytes and phanerogams in a German inland sand ecosystem (Langhans et al. 2010).

4.5.3 Development of chlorophyll *a* content

The chlorophyll *a* content increased, as expected, from the bare-sand status to the formation of an initial crust. The chlorophyll *a* content measured at the beginning of our experiment in bare sand approximately corresponds to the content determined by Brankatschk et al. (2013) in the mobile part of a sand dune. The measured contents after one year of crust development are relatively low compared to initial soil-crust stages of the donor site, where chlorophyll *a* contents of around 114 mg m^{-2} were measured (Langhans et al. 2009a); indeed, these initial crusts were at least five years old. For crusts containing cyanobacteria and eukaryotic algae, Lange (2001) described maximal chlorophyll contents below 100 mg m^{-2}; in bryophyte or lichen crusts the chlorophyll content can exceed 900 mg m^{-2}. In our one-year-old initial crusts, bryophytes had very low cover and as we removed the macroscopic moss plants prior to measurement, bryophytes should not have contributed much to the determined contents (except for occurring protonemata, which were not removed).

Dojani et al. (2011) reported a continuous increase of chlorophyll *a* content during 32 months after disturbance. Therefore, the slight decrease in chlorophyll *a* content after two years is astonishing, especially as the bryophyte cover increased by that time. An explanation could be that the weather was much cooler and less sunny than the previous years (see section 4.3.1 ´Study site´).

4.6 Conclusion

We were able to show that in our study system the approach of transplanting soil-crust pieces to an undeveloped, bare sand site to facilitate the spread of crust organisms is strictly taxa-dependent. There were only slight effects regarding the transfer of cyanobacteria and eukaryotic algae, as these organisms colonized even our newly created dune complex rapidly by airborne dispersal. The transplantation of crust pieces is more promising for the transfer of sand- and dry-grassland-specific bryophytes, and can be used to build up founder populations for further spread of these taxa.

5 Twelve years of fertilization in sandy grassland: Impacts on phytomass, nutrient concentrations and N:P ratios

Cladonia rangiformis/rei

Experimental block; in front a C-addition plot with high cryptogam cover, on the right the NPKM treatment dominated by *Ononis repens* s.l.

Allium sphaerocephalon

5.1 Abstract

Questions: What are the effects on phytomass of different plant functional types after a prolonged period of nutrient additions in nutrient-poor sandy grassland? Which changes of nutrient status in plant tissues are induced by nutrient addition? What is the N:P ratio in sandy grassland and is it suitable for assessing the type of nutrient limitation? Can Ellenberg indicator values indicate changes of site conditions due to nutrient additions in our study system? Do the alterations in successional pathways proceed?

Methods: A five-fold replicated randomised block design with eight treatment types was started in 2000, applying organic carbon (C), phosphorus (P), low- and high-dose nitrogen (N) and combinations of high-dose N and P (NP), potassium (NPK) and other essential nutrients (NPKM). Data were analysed by mixed linear models, and mean Ellenberg indicator values were calculated for each treatment. Successional pathways were analysed using detrended correspondence analysis (DCA).

Results: Already in the year of starting the fertilization, the P concentration in above-ground phytomass of phanerogams, cryptogams and in litter increased following treatments comprising phosphorus; in roots the increase was significant since the second year. Simultaneously, the N:P ratio separated into treatments with and without P, whereof the P treatments had lower N:P ratios. N concentrations were significantly increased by N addition except for roots; most pronounced was the increase in cryptogams (1.4-fold) and litter (1.7-fold). Above-ground phytomass of phanerogams was significantly affected by N addition but not before the third treatment-year; on high-dose N plots phanerogam phytomass and litter accumulation increased 1.9- to 2.5-fold, respectively. In contrast, cryptogam phytomass increased 1.7-fold in treatments without/low-dose N addition. Both phytomass and N:P ratios indicated N as the limiting factor in our ecosystem. The Ellenberg indicator value (EIV) for nutrients revealed the strongest effect of fertilization, increasing under N addition. Also the EIV for moisture increased in high-dose N treatments, whereas the EIV for light diminished. DCA revealed that the separation into two successional pathways proceeded; high-dose N treatments developed on other than the typical pathway.

5.2 Introduction

Fertilization experiments are widely used in vegetation ecology to determine nutrient limitation (Vitousek & Howarth 1991) or threats by enhanced nutrient availability (Phoenix et al. 2012). The limiting nutrient in most terrestrial ecosystems is considered to be nitrogen (N; LeBauer & Treseder 2008). However, in European grasslands mostly co-limitation by N and phosphorus (P) has been observed in fertilization experiments (Morecroft et al. 1994; Niinemets & Kull 2005; Wassen et al. 2005; Hejcman et al. 2007).

Enhanced availability of either N or P or both are threats to existing ecosystems, especially for those adapted to low fertility. Agricultural intensification and combustion processes increased in particular the atmospheric deposition of N in the last century (Galloway et al. 2008); the man-made deposition of P increased as well (Smil 2000). Loss of diversity, especially of endangered species, is one of the major threats of both elevated levels of N (Clark & Tilman 2008; Stevens et al. 2010) and of P (Hejcman et al. 2010a). In addition, shifts in nutrient supply can alter limitation and change floristic composition (Güsewell 2004; Pierik et al. 2011); this can be reflected by shifts in Ellenberg indicator values (Chytrý et al. 2009). In P-limited ecosystems P addition increased total biomass (Carroll et al. 2003; Hejcman et al. 2007); the same applied to N addition in N-limited sites (Gough et al. 2000). Enhanced biomass production after addition of a certain nutrient is a key indicator for nutrient limitation. But, as noted by Aerts & Chapin (2000), results of fertilization experiments sometimes can be difficult to interpret because vegetation does not always respond to nutrient addition.

Another possibility to assess nutrient limitation apart from experimental fertilization studies is the use of nutrient ratios (N:P, N:K and P:K; Hejcman et al. 2010b). The most commonly used is the N:P ratio, generally determined by the ratio of N to P concentrations in above-ground phytomass of phanerogams. Koerselman & Meuleman (1996) concluded, after comparing 40 nutrient addition experiments in European wetlands, that N limitation is given at a ratio of N:P < 14, P limitation at N:P > 16, and N:P values between 14 and 16 indicates co-limitation of N and P. In contrast, Güsewell (2004) proposed N:P ratios below 10 to be indicative for N limitation and above 20 for P limitation; in between this range, no unequivocal relation of fertilization effects and N:P ratios can be demonstrated. The broader range of thresholds suggested by Güsewell (2004) is caused by the fact that the different reviewed ecosystems showed no consistent thresholds for N:P ratios; there was only a general consensus that low N:P ratios indicate N limitation. Nevertheless, Li et al. (2011) suggested that the critical N:P

ratios according to Koerselman & Meuleman (1996) [and presumably as well those of Güsewell (2004)] were not valid for a Chinese semi-arid sandy grassland, where probably lower thresholds for nutrient limitation exist. This illustrates the need for more research to establish thresholds for different ecosystem types.

N:P ratios of cryptogams are even more scarce, especially on the ecosystem level. Most investigations were done for single species only (Arroniz-Crespo et al. 2008; Hogan et al. 2010). As far as we know, only one study related the N:P ratio of mosses to that of vascular plants (Niinemets & Kull 2005). No N:P ratios but at least nutrient concentrations of bryophytes can be linked to vascular plants´ element concentrations in the long-term-fertilized Rengen Grassland Experiment in western Germany (Hejcman et al. 2010b; Hejcman et al. 2010c).

In this paper, we present the results of a 13-yr field study on nutrient additions in low-productive sandy grassland of central Germany. Hereby the investigations of Storm & Süss (2008) and Faust et al. (2012) are complemented; the first focussed on primary production (until 2005) and phytodiversity, and the second on community structure, plant strategies and successional trends.

The aims of the present study were:

(1) to assess the type of nutrient limitation in our ecosystem after a prolonged period of nutrient additions;
(2) to analyse the changes in nutrient status in plant tissues induced by nutrient additions;
(3) to assess the suitability of N:P ratios as indicators of the type of nutrient limitation; and
(4) to validate the ability of Ellenberg indicator values to indicate changes of site conditions due to nutrient additions.

5.3 Methods

5.3.1 Study area

The study area ´Ehemaliger August Euler-Flugplatz von Darmstadt´ is located in the northern Upper Rhine Valley, ca. 30 km south of Frankfurt/Main near Darmstadt (8°35´ E, 49°51´ N). Since 1996 the area has been designated a nature-protection site. The part where our study was conducted occupied sandy grasslands of the Koelerion glaucae complex, which are protected by the European Fauna-Flora-Habitat directive (Natura-2000 Code 6120; Ssymank et al. 1998). The soil type is calcaric Arenosol with silt and clay content of < 10 %. Soil contents of organic carbon (0.7 ± 0.2 %; mean ± SD), total nitrogen (0.7 ± 0.2 mg g^{-1}) and extractable phosphate (14.9 ± 7.9 mg kg^{-1}) are very low (Bergmann 2004). Deposition of airborne nitrogen (wet and dry deposition) was about 16.6 kg ha^{-1} yr^{-1} in 2001/02 (Bergmann 2004) and about 17.2 kg ha^{-1} yr^{-1} in 2009 (Faust 2011). The mean annual temperature during the experiment was 11.0 °C and the mean annual precipitation was 621 mm (data from Frankfurt airport, 2000-2012, Deutscher Wetterdienst, www.dwd.de).

5.3.2 Experimental design

In 2000, five blocks were established in a randomized block design, each containing eight treatment-plots of 11.56 m^2. The plots were separated by 50-cm-wide paths. Each plot consisted of a 4-m^2 permanent plot for vegetation relevés, and the surrounding area was used for phytomass and soil sampling. Fencing protected the plots from grazing by sheep and rabbits.

In June 2001 the treatment of the plots started. Ten times a year the different treatment approaches were applied to the plots dissolved in 10 l tap water. The treatments were as follows: control plot (0, only tap water), organic carbon (C, sucrose and once a year sawdust), nitrogen in low (n, 25 kg ha^{-1} yr^{-1}) and high (N, 100 kg ha^{-1} yr^{-1}) dose, phosphate (P, 50 kg ha^{-1} yr^{-1}), and combined nutrients NP, NPK (+ 60 kg ha^{-1} yr^{-1} potassium) and NPKM (+ other essential nutrients). For more details concerning exact dosages and compositions of the treatments see Storm & Süss (2008).

5.3.3 Soil analysis

Soil samples were taken in 2000 and since 2001 in a biannual cycle using a Pürckhauer soil corer (inner diameter 20 mm; Windaus, Clausthal-Zellerfeld, DE). Per subplot two soil samples were taken of 0-10 cm depth and bulked to two composite samples per plot. The samples were kept cool, sieved (2 mm) within 24 h and frozen (-18 °C) until extraction. The thawed samples were analysed for plant-available nitrate, ammonium and phosphate. For nitrate and ammonium analysis calcium chloride extracts (0.0125 mol l^{-1}, 50 g soil + 200 ml) were prepared. Phosphorus was extracted in 0.05 mol l^{-1} calcium acetate, 0.05 mol l^{-1} calcium lactate and 0.3 mol l^{-1} acetic acid (10 g soil + 200 ml; VDLUFA 1991). These analyses were carried out photometrically (Segmented Flow Analyser SAN+, Skalar Analytical, AA Breda, NL; nitrate: hydrazine reduction, coupling to azo dye; ammonium: Berthelot reaction; phosphate: antimony-phospho-molybdate complex). Potassium was analysed by atomic absorption spectrometry (AAnalyst 300, Perkin Elmer, Waltham, MA, USA). Water content was determined on parallel subsamples of the soil; all nutrient concentrations were converted to oven dry soil (70 °C). pH values were measured in 0.01 mol l^{-1} calcium chloride (pH meter: WTW, Weilburg, DE; glass electrode: Inlab 412, Mettler-Toledo, Greifensee, CH). All analyses were run in duplicate and mean values were taken.

5.3.4 Phytomass sampling and nutrient analysis

Phytomass sampling was carried out yearly during the peak standing crop period in September (2000-2011), at least four weeks after the previous fertilization. Peak-standing-crop data were used to estimate annual productivity (even though actual productivity is underestimated), as this method is frequently used (Gough et al. 2000; Osem et al. 2004). Per plot six randomly chosen subplots (each 400 cm^2) were sampled and bulked together for each plot. Above-ground vegetation (including standing dead) was clipped at ground level and phytomass was separated into the plant functional types (PFTs) ´forbs + graminoids´, ´legumes´ and ´cryptogams´; since 2006 bryophytes and lichens were analysed separately. Additionally litter was collected. Because of erratic occurrence with little phytomass, legumes were included in the PFT ´forbs + graminoids´ for phytomass weights but not for nutrient analysis. To obtain the below-ground phytomass (roots and rhizomes, in the following denoted as ´roots´; up to 30 cm depth) a liner sampler (4.7 cm diameter, Eijkelkamp, Giesbeek, NL) was used (one sample per subplot). Per plot the samples were bulked; cryptogams were cleaned from adhesive sand; below-ground samples were rinsed with tap

water through a sieve (0.71 mm) to extract roots from the soil. All samples were dried at 70 °C and weighted.

N and C concentrations in the phytomass were analysed by elemental analysis (gas-chromatography; Model 1400, Carlo-Erba, Milan, IT). P concentration was determined by a modified Kjeldahl digestion of the samples followed by photometric analysis (molybdenum blue method). Accuracy was ascertained by certified material (Hay powder BCR 129 by LGC Standards, Teddington, UK; N: 98.8 ± 1.5 %, n = 145, P: 97.3 ± 2.5, n = 80, mean ± SD). All analyses were carried out twice. Precision was determined by mean absolute deviation from the mean of both values (N: 1.4 ± 1.5 %, P: 2.0 ± 2.7 %, n = 2209, mean ± SD). To account for contaminations of the samples by adhering sand, N and P concentrations are given for the dry organic matter. Organic matter was calculated by multiplication of the C concentration by 2.02. This factor was determined by dry ashing (550 °C, 4 hours) and parallel C analysis of 102 phytomass samples (SE = 0.01).

5.3.5 Data analysis

N and P pools in phanerogams (above-ground + roots) were calculated as N_{pool} [kg ha^{-1}] = phytomass [g m^{-2}] * N-concentration [%] * 10 and accordingly P_{pool} [kg ha^{-1}] = phytomass [g m^{-2}] * P-concentration [mg P g^{-1}] * 10. Ellenberg indicator values (EIVs) for nutrients, soil reaction, moisture and light were calculated as unweighted means of indicator values for species present in the treatments using ELLEX 2005 (ecosurvey büro bremen, Bremen, DE).

Prior to statistical analysis weighted averages had to be calculated for some variables; these variables were the nutrient contents of ´forbs + graminoids´ (in 2000 and 2001 separately analysed) and of cryptogams (averaged of bryophytes and lichens, in the years 2006-2010).

Mixed linear models (SAS 9.2, PROC MIXED; SAS Institute Inc., Cary, NC, USA; Littell et al. 2006) were used to analyse the effects of the variables ´treatment´, ´treatment-group´ and ´year´ on the dependent variables, being suitable especially for analysis of repeated-measures data (Littell et al. 1998). According to the corrected Akaike criterion (AICC) 14 covariance structures were compared (Fernández 2007). Thereof the simpler structure was chosen, when two covariance structures led to equal AICC values. The Kenward-Roger approximation was selected to calculate the degrees of freedom (Schaalje et al. 2002).

Dunnett-adjusted post-hoc tests were conducted to compare all treatment types with the control using the LSMEANS procedure of PROC MIXED. Thereafter, following Storm & Süss

(2008) and Faust et al. (2012), for some analyses the treatments were pooled into two treatment groups (N–: 0, C, P, n; N+: N, NP, NPK, NPKM) and the ESTIMATE procedure was used for further analysis. To apply the ´baseline´ option of PROC MIXED, the year 2000 was used as covariate for every following year. To determine differences between the treatment groups within the investigated years Tukey-adjusted post-hoc tests were carried out. Significance was set at a level of $P < 0.05$.

Community composition was analysed with detrended correspondence analysis (DCA) using PC-Ord 6.09 (MjM Software, Gleneden Beach, OR, USA). Means of the root-transformed cover values of the five replicates were used. The analysis was run using the options ´downweight rare species´ and ´rescale axes´; the number of segments was 26.

5.4 Results

5.4.1 Nutrient concentrations in soil

Plant available nitrogen (N_{min}) was not affected by any treatment until 2004; since then, the high-dose N treatment group had 1.5 to 1.8-fold higher N_{min} concentrations than the N– group (Fig. 5.1 a). Extractable phosphate accumulated on plots treated with P-containing fertilizer (Fig. 5.1 b). In 2010, the increase was 8-fold compared to the control. The concentration of potassium increased on NPK and NPKM plots, reaching values 5-fold higher than in the control (2010; Fig. 5.1 c). pH values decreased on all plots (control: pH 7.6 in 2000, pH 7.3 in 2010), the strongest decline was found on NPK and NPKM plots (pH 7.6 in 2000, pH 7.1 in 2010).

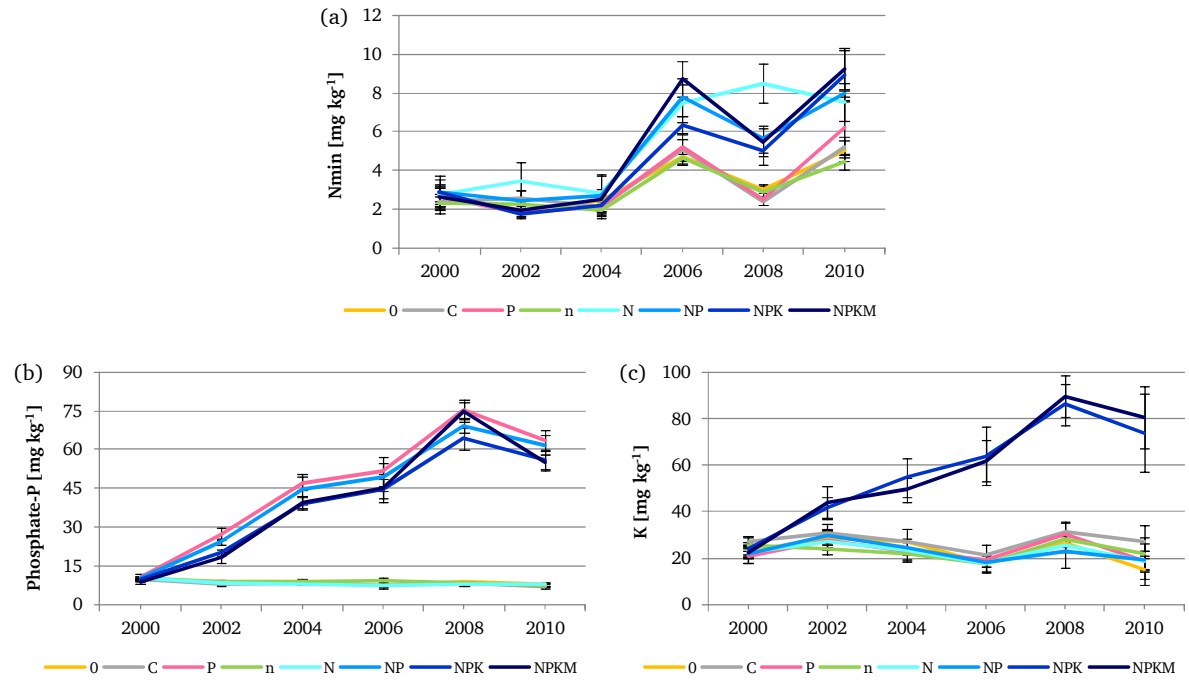


Fig. 5.1: Nutrient concentrations in the soil of the different treatment plots (mean ± SE; n = 5).

5.4.2 Phytomass and litter

All analysed phytomass variables show significant effects of treatment and year (Table 5.1). For above-ground phytomass of phanerogams, all high-dose N treatments increased significantly compared to the control; low-dose N, C and P treatments did not differ from the control (Fig. 5.2 a). With regard to roots no individual treatment was significantly different from the control, but the high-dose N treatments achieved higher phytomass values than the low-dose N treatments (Fig. 5.2 b). For cryptogams the only significant effect was a progressive decrease of phytomass under NPKM addition in comparison to the control; the NPKM addition lowered phytomass up to 3.8-fold in 2011 (Fig. 5.2 c). The other high-dose N treatments decreased cryptogam phytomass as well, although not significantly. Litter significantly increased on all high-dose N treatments, especially by NPKM addition; the other treatments were not significantly different from the control (Fig. 5.2 d). In none of the analysed variables did the addition of carbon or P have a significant effect on phytomass or litter.

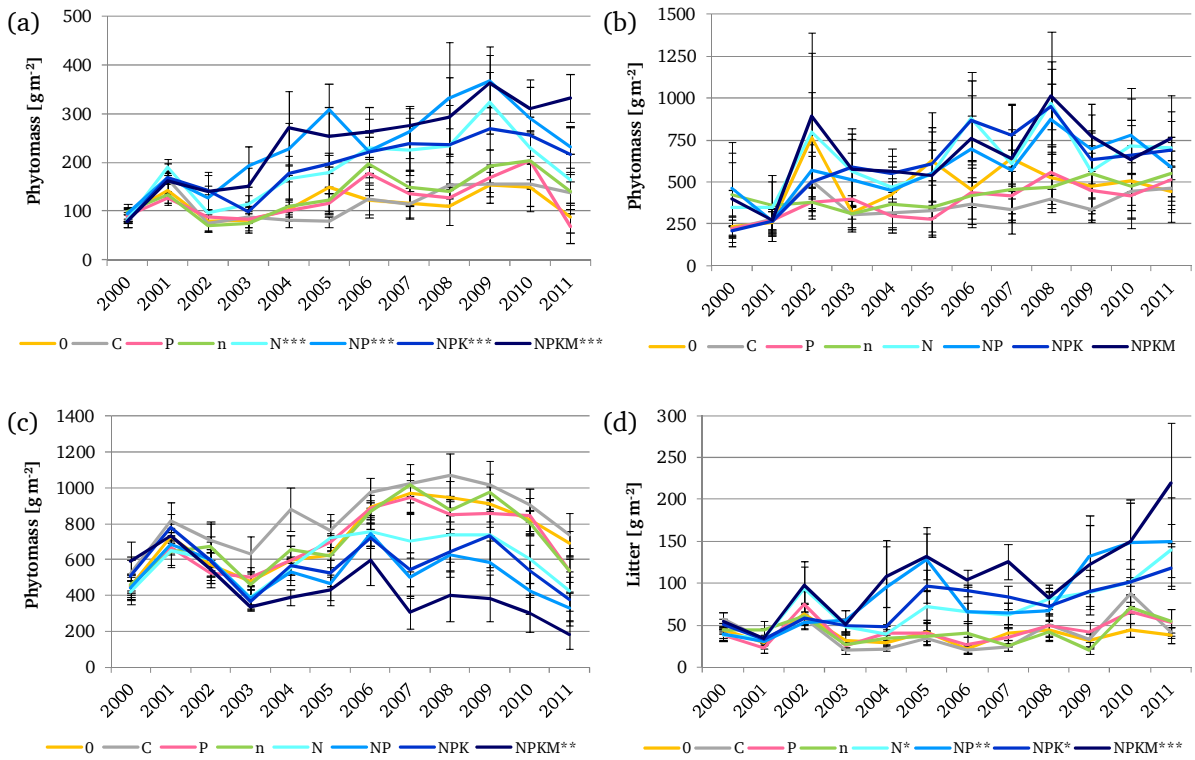


Fig. 5.2: Phytomass production of the eight treatment types (mean ± SE; n = 5). (a) above-ground phytomass of phanerogams; (b) phytomass of roots; (c) phytomass of cryptogams; (d) mass of litter. Treatment effects that are significantly different from the control are marked with asterisks.

Table 5.1: Results of the linear mixed models for the analysed variables of the eight individual treatments. Significant results are shown in bold print. bl = baseline; P = level of significance. Arrows indicate an increase (↗) or decrease (↘) in the N+ group or, according to P-content and N:P ratio, in the P+ group. ´phanerogams´ implies the above-ground parts.

	treat	**year**	**treat*year**	**bl**	**N+N-**	**direction**
Variable	*P*	*P*	*P*	*P*	*P*	**of effect**
Phytomass						
phanerogams	**<.0001**	**<.0001**	0.5821	0.2719	**<.0001**	↗
roots	**0.0381**	**<.0001**	0.5888	**0.0252**	**0.0009**	↗
cryptogams	**0.0006**	**<.0001**	0.0732	0.8739	**<.0001**	↘
litter	**<.0001**	**<.0001**	**0.0024**	0.8218	**<.0001**	↗
N-concentration						
phanerogams	**<.0001**	**<.0001**	**0.0038**	0.2492	**<.0001**	↗
roots	**0.0022**	**<.0001**	0.6049	0.3624	**0.0002**	↗
cryptogams	**<.0001**	**<.0001**	0.3326	**0.0004**	**<.0001**	↗
litter	**<.0001**	**<.0001**	**0.0020**	0.2128	**<.0001**	↗
P- concentration						
phanerogams	**<.0001**	**<.0001**	**0.0015**	0.8493	**<.0001**	↗
roots	**<.0001**	**<.0001**	**<.0001**	0.9873	**<.0001**	↗
cryptogams	**<.0001**	**<.0001**	**<.0001**	0.2143	**<.0001**	↗
litter	**<.0001**	**<.0001**	**0.0046**	0.1695	**<.0001**	↗
N:P ratio						
phanerogams	**<.0001**	**<.0001**	**0.0066**	**0.0032**	**0.0255**	↘
roots	**<.0001**	**<.0001**	**0.0112**	0.0766	**0.0022**	↘
cryptogams	**<.0001**	**<.0001**	**0.0400**	**0.0082**	**<.0001**	↘
litter	**<.0001**	**<.0001**	**0.0297**	**0.0024**	**<.0001**	↘
Ellenberg indicator value						
N (nutrients)	**0.0046**	**<.0001**	0.5599	0.0825	**0.0002**	↗
F (moisture)	0.1093	**0.0017**	0.9878	0.1329	**0.0044**	↗
L (light)	0.2261	**<.0001**	0.7885	**0.0164**	**0.0436**	↘
R (soil reaction)	0.1441	**<.0001**	0.7607	**<.0001**	0.2171	–

Pooling into treatment-groups resulted in a significant separation of low- and high-dose N treatments in all analysed variables (Table 5.2). Since the second year of fertilization above-ground phytomass of phanerogams was significantly higher in the N+ group compared to the N– group; the increase was about 1.9-fold and remained approximately stable since the third year of nutrient additions. The increase in phytomass of roots in the N+ group was about 1.6-fold in comparison to the N– group. Significantly more roots´ phytomass was found in the N+ group in the years 2003, 2006 and since 2008. In contrast to the other PFTs, cryptogams achieved significantly higher phytomass values in the N– group since 2003; the N+ group remained approximately stable with fluctuations until 2009 and decreased since then. Since 2007 about 1.7-fold more phytomass was determined in the N– group than in the N+ group. Litter production remained nearly stable in the N– group with a slight increase in the last two years, whereas litter increased significantly in the high-dose N group since 2003. Since about 2004/05 the increase of litter was fluctuating around a 2.5-fold increase in the N+ group.

Tab. 5.2: Results of the linear mixed models for the analysed variables of the treatment-groups (tg) N– and N+. Significant results are shown in bold. P = level of significance. ´phanerogams´ implies the above-ground parts.

	tg	**year**	**tg*year**	**tg*year 2001/02**	**tg*year 2002/03**	**tg*year 2003/04**	**tg*year 2004/05**	**tg*year 2005/06**	**tg*year 2006/07**	**tg*year 2007/08**	**tg*year 2008/09**	**tg*year 2009/10**	**tg*year 2010/11**	**tg*year 2011/12**
Variable	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*
Phytomass														
phanerogams	**<.0001**	**<.0001**	**0.0024**	0.1122	**0.0037**	**0.0214**	**<.0001**	**<.0001**	**0.0041**	**<.0001**	**0.0002**	**<.0001**	**0.0012**	**<.0001**
roots	**0.0003**	**<.0001**	**0.0070**	0.7616	0.0606	**0.0164**	0.1102	0.0921	**<.0001**	0.0562	**<.0001**	**0.0259**	**0.0127**	**0.0443**
cryptogams	**<.0001**	**<.0001**	**<.0001**	0.9795	0.6376	**0.0275**	**0.0117**	**0.0423**	**0.0032**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
litter	**<.0001**	**<.0001**	**<.0001**	0.8990	0.3328	**<.0001**	0.0231	**<.0001**	**<.0001**	**<.0001**	**0.0043**	**0.0002**	**0.0063**	**<.0001**
Ellenberg indicator value														
N (nutrients)	**<.0001**	**<.0001**	**0.0217**	0.6539	0.7053	0.1502	**0.0405**	**0.0016**	**0.0490**	**0.0067**	**0.0435**	**0.0028**	**0.0014**	**<.0001**
F (moisture)	**0.0066**	**0.0015**	0.4913	0.1569	0.8855	0.1848	**0.0316**	**0.0070**	0.0722	0.2741	0.4764	0.0701	0.0889	**0.0063**
L (light)	**0.0357**	**<.0001**	0.4866	0.3526	0.2692	0.1137	0.5125	0.1863	0.3675	**0.0482**	0.6188	0.9085	**0.0312**	**0.0380**
R (soil reaction)	0.2009	**<.0001**	**0.0409**	0.3862	0.8121	0.2758	**0.0162**	0.7913	0.2238	0.1176	0.0625	0.7913	0.6780	0.1568

5.4.3 Nitrogen concentration in phytomass and litter

A significant effect of treatment and year is shown for all variables (Table 5.1). Regarding the above-ground phytomass of phanerogams, the high-dose N treatments had significantly higher N concentrations than the control; C, P and low-dose N addition had no significant effect on N concentration (Fig. 5.3 a). The roots´ N concentration did not show significant differences between control and any other treatment (Fig. 5.3 b). In cryptogams, all N-addition treatments, including the low-dose N treatment, resulted in a significant increase of N concentration compared to the control (Fig. 5.3 c). C addition reduced the N concentration in cryptogams, although not significantly. The N concentration in litter significantly increased in the NP, NPK and NPKM treatments; the other treatments did not show significant differences from the control (Fig. 5.3 d).

For all variables pooling into N–/N+ treatment groups induced a significant effect (Table 5.1). Already since the first year of fertilization the N+ group in above-ground phytomass of phanerogams had on average (with fluctuations) about 1.2-fold higher N concentrations than the N– group. For roots, the mean increase of N concentration in the N+ group was around 1.1-fold compared to the N– group since 2003. The cryptogams´ N concentration increased in the N+ group 1.2-fold (2003-2007) in comparison to the N– group and increased up to 1.4-fold in the last year. In litter, the N concentration increased progressively in the N+ group, reaching 1.6-fold higher N concentrations than in the N– group in 2010.

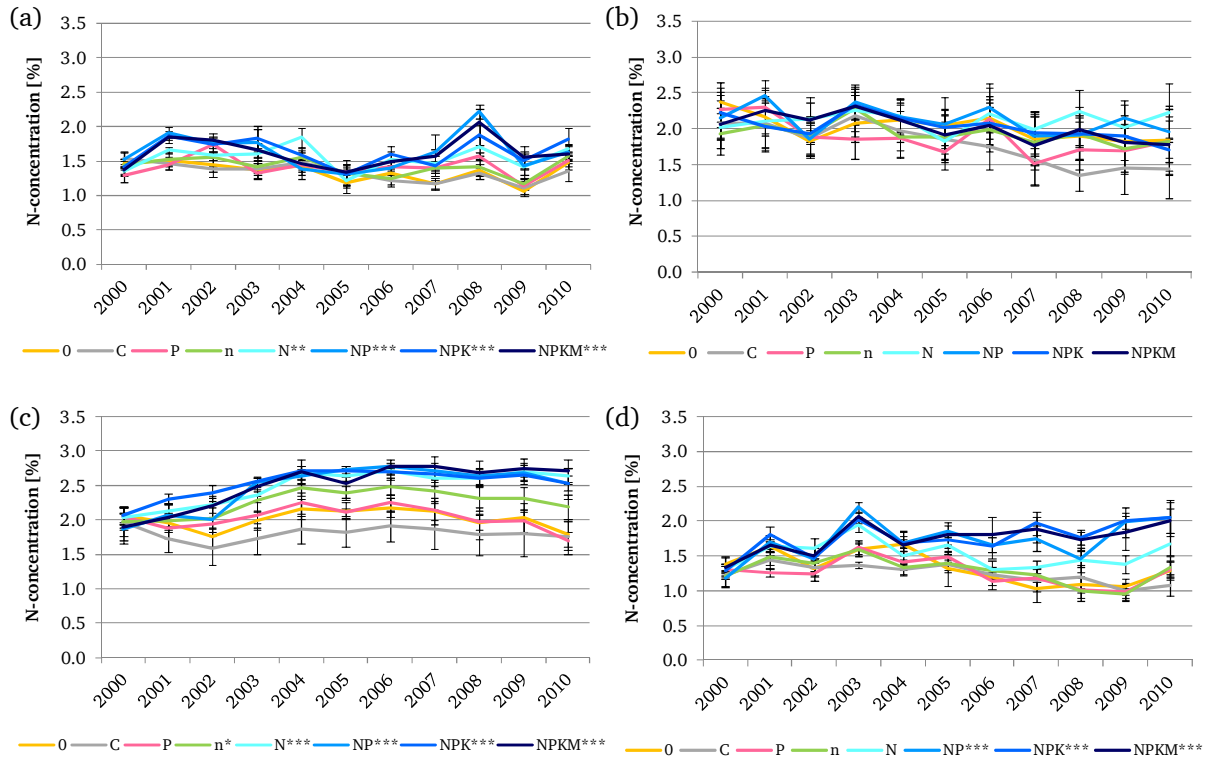


Fig. 5.3: N-concentrations of the different treatment types (mean ± SE; n = 5). (a) above-ground phanerogams; (b) roots; (c) cryptogams; (d) litter. Treatment effects that are significantly different from the control are marked with asterisks.

5.4.4 Phosphorus concentration in phytomass and litter

All analysed variables show a significant effect of treatment and year (Table 5.1). In treatments with addition of P-containing fertilizer (P+) the P concentration increased highly significantly in all analysed variables, whereas C, low- and high-dose N treatments did not differ significantly from the control (P–; Fig. 5.4 a-d).

Comparing the P– and P+ treatment-groups, in the P+ group the P concentration in the above-ground phytomass of phanerogams and of roots continuously increased up to 2.3-fold and 2.2-fold in 2009, respectively, and slightly decreased in 2010. In cryptogams, the P concentration increased until 2003 and since then remained stable at a 1.9-fold higher P concentration than in the P– group. The P concentration in litter increased in the P+ group by 2.3-fold compared to the P– group and since 2005 fluctuated around this value.

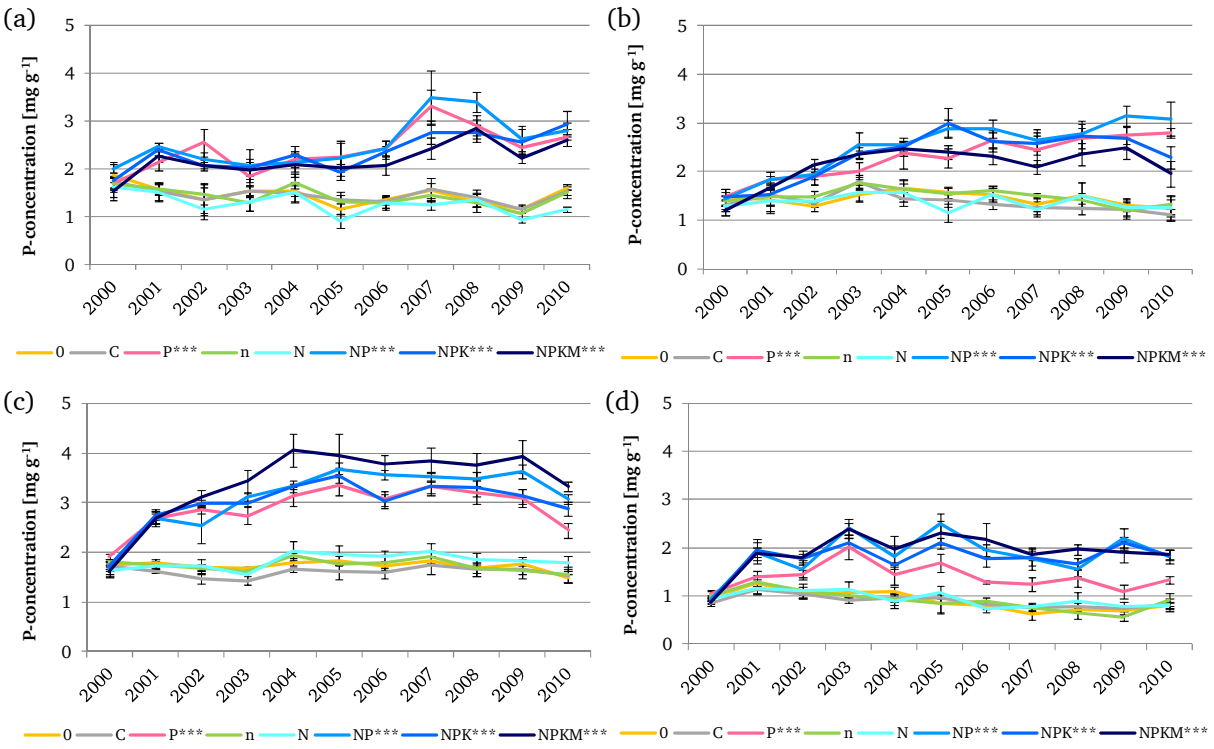


Fig. 5.4: P-concentrations of the different treatment types (mean ± SE; n = 5). (a) above-ground phanerogams; (b) roots; (c) cryptogams; (d) litter. Treatment effects that are significantly different from the control are marked with asterisks.

5.4.5 N and P pool

Addition of N in high dose resulted in an increase of the N pool in phytomass of phanerogams until 2006. Since then, the N pool of the N+ group was fluctuating around 100 kg N ha^{-1} above the N pool in the N– group, which corresponds to the annual amount of N applied with fertilizer (100 kg N ha^{-1} yr^{-1}).

The P pool in phytomass of phanerogams progressively increased with P addition in comparison to the P– group. Since 2007, the P+ group had about 16 kg P ha^{-1} more than the P– group, which is about one third of the yearly applied phosphorus (50 kg P ha^{-1} yr^{-1}).

5.4.6 N:P ratio

For the N:P ratio significant effects of treatment and year are evident for all variables (Table 5.1). The control had a relatively constant N:P ratio of around 10 in above-ground phytomass of phanerogams (Fig. 5.5 a). The N:P ratio significantly increased in the high-dose N treatment (2010: 15), while it significantly decreased on sites treated with P addition

compared to the control (2010: on average 6). Low-dose N and C addition treatments were not significantly different from the control. In roots, the P addition treatments had significantly lower N:P ratios than the control; the other treatments did not differ from the control significantly (Fig. 5.5 b). The N:P ratio of cryptogams declined significantly in all P treatments compared to the control (Fig. 5.5 c). Although not significant, both treatments with N in low- and high-dose had higher N:P ratios than the control; C addition had no significant effect on the N:P ratio of cryptogams. Also in litter, the P addition treatments achieved significantly lower N:P ratios than the control, whereas the other treatments did not differ significantly (Fig. 5.5 d).

For the N:P ratios it is suitable to pool into P– and P+ groups, because all treatments with P addition were grouped together. The P+ group achieved lower N:P ratios than the P– group in all variables. In above-ground phytomass of phanerogams the N:P ratio constantly decreased in P+ group to about 50 % of the P– group in 2011. In roots the decline was about 50 % in the P+ group compared to the P– group until 2005 and remained stable since then. In cryptogams and litter the N:P ratio declined in the P+ group to about 60 % of the P– group in 2003, and since then the decrease remained stable.

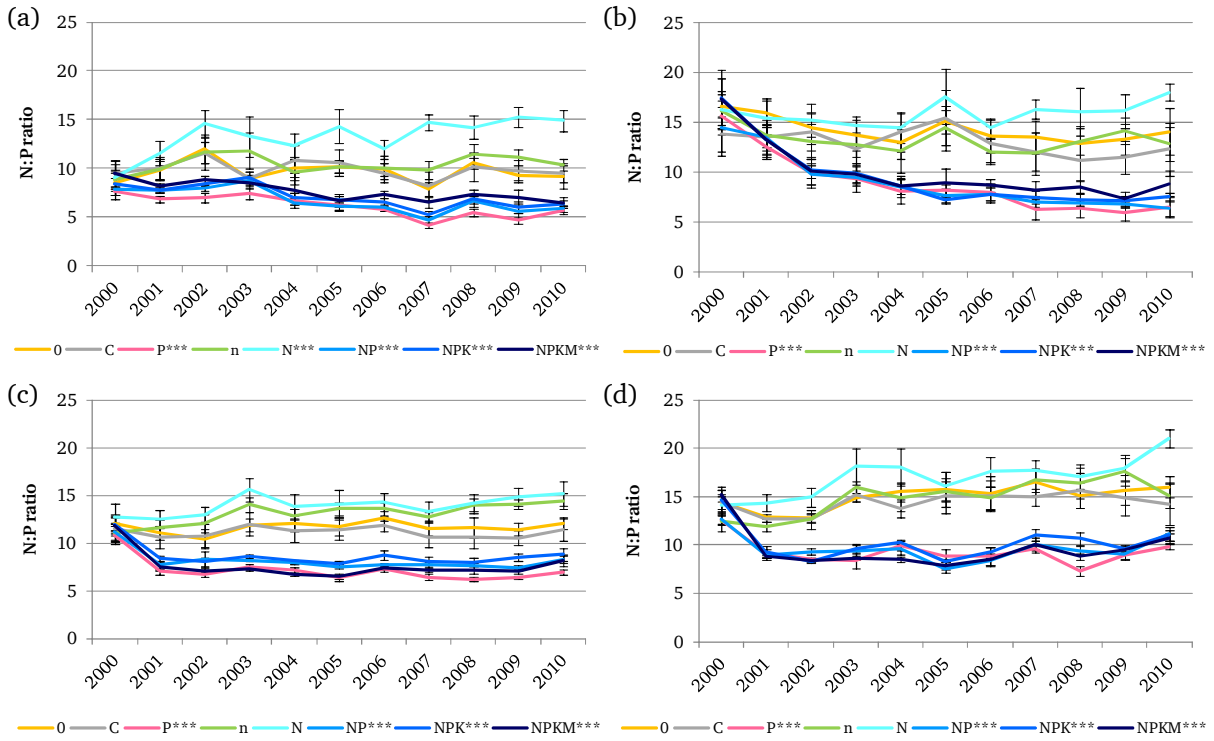


Fig. 5.5: N:P ratio of the different treatment types (mean ± SE; n = 5). (a) above-ground phanerogams; (b) roots; (c) cryptogams; (d) litter. Treatment effects that are significantly different from the control are marked with asterisks.

5.4.7 Ellenberg indicator values

For all investigated EIVs the effect of year was significant and in case of the nutrient value also the treatment. The EIV for nutrients increased in all high-dose N treatments; since 2005/06 the values were almost always higher than the control (Fig. 5.6 a). However, only in the NPKM treatment was the increase significant, with the N value rising from 2.9 ± 0.1 (mean ± SE) in 2000/01 to 3.8 ± 0.2 in the last year. The EIV for moisture increased and separated from the control especially in the NPKM treatment; for the other high-dose N treatments this trend was less distinct (Fig. 5.6 b). The EIV for light diminished since 2006/07 in all treatments, but this decline was more pronounced in NP, NPK and NPKM treatments (Fig. 5.6 c).

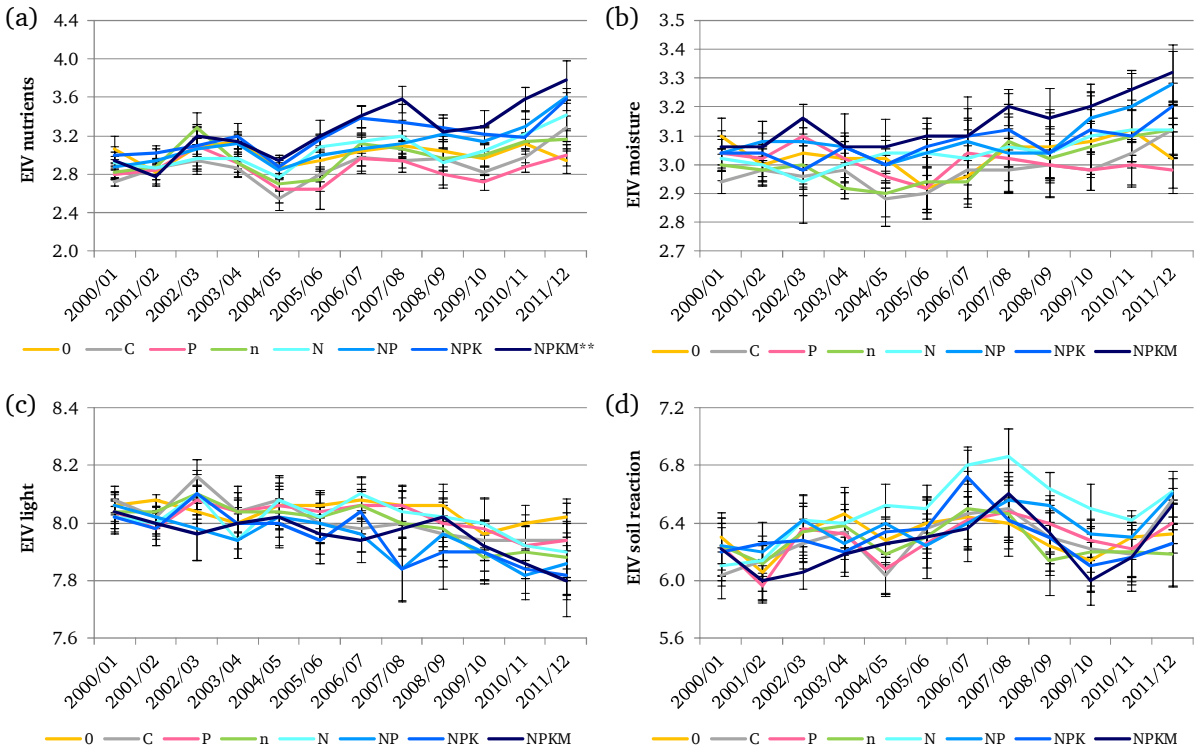


Fig. 5.6: Mean Ellenberg indicator values for the different treatment types (mean ± SE; n = 5). Treatment effects that are significantly different from the control are marked with asterisks.

Pooling into treatment groups resulted in a significant separation of N– and N+ groups in EIVs for nutrients, moisture and light (Table 5.2). Since 2004/05 the nutrient value was significantly higher in the N+ group than in the N– group (2011/12: 0.5 units). The EIV for moisture also had higher values in the N+ group compared to the N– group, but only for the years 2004/05, 2005/06 and 2011/12 (0.2 units) was this increase significant. The light value

decreased in the N+ group especially in the last years, with a significant decline in 2007/08, 2010/11 and 2011/12 (0.1 units).

The EIV for soil reaction did not show a separation of the treatments or the treatment groups (Fig. 5.6 d).

5.4.8 Successional pathways

During the first two years the eight treatments can hardly be separated (Fig. 5.7). Along the first axis all treatments develop in the same direction, but since the second year of nutrient addition the trajectories of the N– and N+ groups are separated along the second axis. This separation became more pronounced since 03/04 and is still in progress. Thus addition of N in high dose served as promoter of succession. Plots with C or P addition proceeded further away from the N+ group than control and low-dose N treatments did.

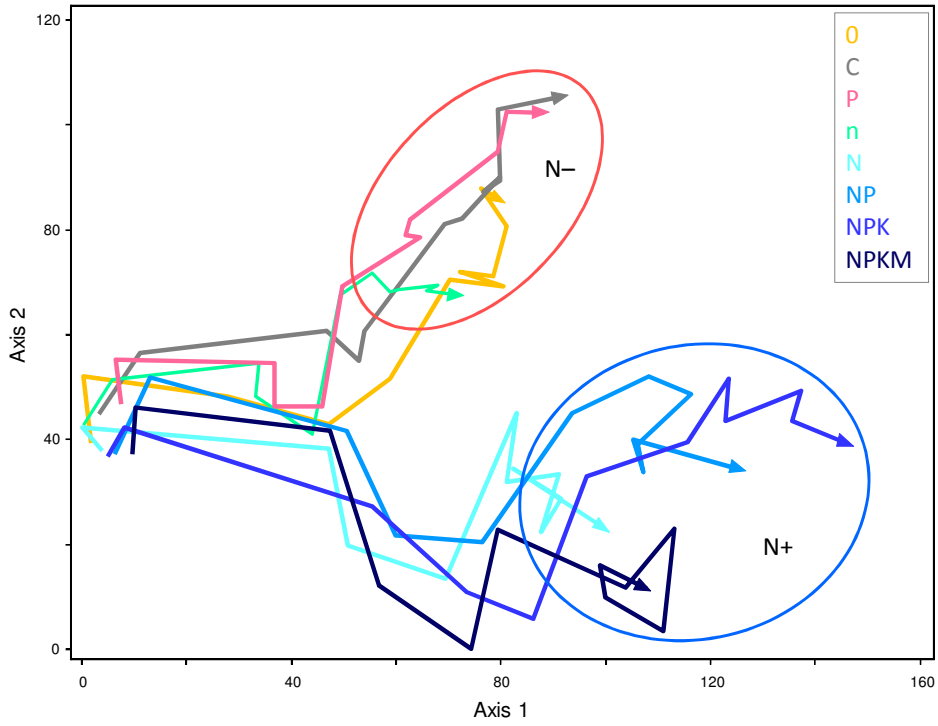


Fig. 5.7: Detrended correspondence analysis (DCA) of the eight treatments types (2000/01-2011/12). Single time points of the treatments are connected by trajectories. Eigenvalues: axis 1: 0.12, axis 2: 0.06, axis 3: 0.04.

5.5 Discussion

5.5.1 Soil

The initial nutrient concentrations in soil were low in all treatments. In our study system mean N_{min} values of 2.6 mg kg^{-1} were reported by Süss et al. (2004) and according to Storm et al. (1998) the P contents are typically below 15-20 mg kg^{-1}. In the first years mineral nitrogen concentration in soil was stable, and it was not until 2006 that N_{min} accumulated in the high-dose N treatments. This lag-phase is probably induced by uptake of the added nitrogen through plants and microorganisms (Storm & Süss 2008). The delayed accumulation can presumably be attributed to mineralization of the then N-enriched litter, releasing the N permanently. The addition of N in low dose had no effect on the mineral nitrogen concentration in soil.

Both phosphorus and potassium accumulated in soil since the first sampling following fertilization. Accumulation of phosphorus in the soil is reported for long-term application of P-containing fertilizer (Schellberg et al. 1999), and the same applies to potassium (Hejcman et al. 2010a). Since 2004, the concentrations of both P and K are in the range of or exceeded 40-50 mg kg^{-1}; above this value both nutrients are not considered to be limiting in agriculture (Scheffer & Schachtschabel 2002).

It can be concluded that the applied fertilizers have raised the soil concentrations of plant-available nutrients to an extent which should be sufficient to overcome nutrient limitations.

5.5.2 Phytomass and litter

Addition of N in high dose (100 kg ha^{-1} yr^{-1}) caused a significant increase in above-ground phytomass of phanerogams, revealing that productivity in this sandy grassland type is N-limited. A co-limitation by P, as reported in other European grasslands (Willems et al. 1993; Morecroft et al. 1994; Niinemets & Kull 2005), could not be observed, as the addition of P alone did not increase phytomass. This became apparent already in the fourth year of nutrient addition, as described by Storm & Süss (2008).

Since then, phytomass in the high-dose N group increased further; however, production is still low (NPKM maximum in 2009: 360 g m^{-2}) compared to, e.g., 700 g m^{-2} in chalk grassland after five years of NPM addition (control: 250-350 g m^{-2}; Willems et al. 1993). Since phytomass in the N– group slightly increased simultaneously, a remarkable effect occurred:

after phytomass nearly doubled in the N+ group compared to the N– group until 2004, the ratio stabilized around this value and has remained stable since then. This indicates that other factors are limiting production besides nutrients. Water as a limiting factor is already discussed by Storm & Süss (2008), just like more time for successional changes. Another eight years of investigations revealed changes in community composition (Faust et al. 2012), but nevertheless, this had little effect on phytomass. Therefore, another probable factor might be dispersal limitation for species with higher productivity and adaptation to nutrient-richer sites. The local seed rain consists mostly of seeds from autochthonous species (Faust et al. 2012).

As pointed out, N is the limiting factor in our system; but N in low dose (25 kg ha^{-1} yr^{-1}) had no significant effect on above-ground phytomass of phanerogams. In other studies the increase of biomass production was dose-related to the N input (Song et al. 2011; Isbell et al. 2013), but the rise in productivity can be slower in low-dose (30 kg ha^{-1} yr^{-1}) than in high-dose (above 60 kg ha^{-1} yr^{-1}) N treatments (Song et al. 2011). Leaching or immobilization of N could explain why no effect of N in low dose was found.

The addition of C-sources did not result in a reduction of above-ground biomass of phanerogams, as observed e.g. by Blumenthal (2009) in a semi-arid mixed-grass prairie and by Spiegelberger et al. (2009) in a mountain grassland; it was not significantly different from the control. A reason could be that the biomass production on the C-addition plots was already very low (2000: 88 ± 3 g m^{-2}); a further reduction may be improbable.

The increase in root biomass under high-dose N addition was not as pronounced as in above-ground phytomass of phanerogams. The outcome of this is a decreased ratio of roots to above-ground phytomass, as reported in other studies (Stevenson 1995; Holub et al. 2013). I have two explanations for this. At first, for the plants´ nutrient requirements a strengthened root growth is non-essential, because the supply of nutrients in the soil is elevated through fertilization. Secondly, it is advantageous for the plants to invest more in above-ground than below-ground parts to have a competitive benefit concerning light and space. As recorded for above-ground phytomass of phanerogams, the N+/N– ratio of phytomass did not change further after 2004.

The phytomass of cryptogams decreased under high-dose N addition, especially under the compound fertilizer treatment (NPKM). A stabilization of this decrease occurred later than in the other functional groups, in 2007. The decrease can particularly be explained by a decline in bryophyte biomass in these treatments (see Faust et al. 2012); bryophytes represent the

greater portion of cryptogam biomass than lichens in our study system (control: in average 90 % bryophytes). Explanations for this decrease could be a direct toxicity of applied fertilizer elements or, more likely, an indirect effect through shading by vascular plants. Competition for light, induced by fertilizer-enhanced growth of vascular plants, can have a strong influence on moss performance even in unproductive low-biomass ecosystems (van der Wal et al. 2005). Both phytomass of phanerogams and mass of litter increased on high-dose N treatments as well as the cover of forbs and graminoids (Faust et al. 2012), probably outcompeting especially bryophytes. Negative correlations were found between vascular plant biomass and bryophyte biomass (Bergamini & Pauli 2001; Bergamini et al. 2001) and between bryophyte biomass and litter production of vascular plants (van der Wal et al. 2005; Hejcman et al. 2010c). Even though lichens occurred only on parts of the plots (hence statistically not evaluable), phytomass production seemed to be facilitated (or at least not negatively affected) by C addition and in tendency by P-containing fertilizers. In both C and P treatments the ratio of lichens in cryptogams´ phytomass increased by 2-fold between 2006 and 2011. Sparrius et al. (2013) observed a general decline of lichen cover by N addition and an increase by P addition, indicating a P limitation of the lichen layer.

The mass of litter increased, as expected, on the N+ treatments (Li et al. 2011). Litter accumulation in the N+ group in relation to the N– group was stronger (2.5-fold) than this ratio in above-ground phytomass of phanerogams (1.9-fold); a more or less stable plateau of this ratio was reached approximately one year later than in phanerogams. This indicates that the decomposition of litter is slower than the production by above-ground phytomass. Additionally, no management (grazing or mowing) was applied, which could have reduced litter production.

5.5.3 Nutrient concentrations and N:P ratios

In above-ground phytomass of phanerogams, addition of N in high dose resulted in a slight increase of the tissue N concentration (cf. Malhi et al. 2010). The increase was less pronounced as investigated by Song et al. (2011) in a Chinese semi-arid grassland (control: around 1 % N; addition of 120 kg N ha^{-1} yr^{-1} for six years: 1.6-2.1 % N), but the measured concentrations were in the same range (control: mean 1.35 % N; N+ group in 2010: 1.68 % N). Addition of N in low dose had no significant effect on N concentration; also in other studies a significant positive relationship between N addition rate and vegetation N concentration was found only at higher N rates (e.g. Song et al. 2011: ≥ 60 kg N ha^{-1} yr^{-1}).

The addition of C sources had no decreasing effect on N concentration, which could have been expected assuming that enhanced microbial activity immobilized soil N, resulting in less plant-available N (reviewed in Perry et al. 2010). Plant-available N was not reduced, as soil data reveal.

The effects of N fertilization were also visible in the development of the N pool in total phanerogam phytomass. During the first years the N pool of the N+ group was increasing until it reached a value corresponding to the N input (100 kg N ha^{-1}) in 2006. We do not assume that the entire added N is taken up by plants, but equilibrium may be reached among N input, loss and mineralization. The steady increase during the first years can be explained by changes in the plant community towards plants with better N exploitation and higher phytomass production, binding more N. Additionally, the mineralization of N from nitrogen-enriched litter commenced with delay, but was then a steady source for additional N.

More pronounced than the increase in tissue N was the increase in P concentration of above-ground phytomass, when P-containing fertilizer was applied. This increase occurred immediately in the first year of application. Hejcman et al. (2010b) recorded as well a positive relationship between P concentration in biomass and P application in a low-productive mountainous hay meadow (control: 1.2 mg P g^{-1}; 67 years of different P treatments: 2.2-3.5 mg P g^{-1}); thus spanning a wider range than the concentrations measured in our system (control: 1.5 mg P g^{-1}; P+ group in 2010: 2.75 mg P g^{-1}). In relation to the unaltered biomass production in the P treatment, it can luxury consumption of P by phanerogams can be assumed, as Bennett & Adams (2001) and Li et al. (2011) did for semi-arid grasslands in Australia and China, respectively.

The P pool increased less pronounced than the N pool; after 2007 the increase stabilized by only about one-third of the annually applied phosphorus (50 kg P ha^{-1} yr^{-1}). One explanation may be that the plant community's requirement for P is already saturated at this level, as P is not a co-limiting nutrient in our system.

The addition of P had a greater influence on N:P ratios of above-ground phytomass of phanerogams than that of N. All treatments with P addition caused a decrease of the N:P ratio (Ludwig et al. 2001; Craine et al. 2008), whereas the addition of N enhanced the N:P ratio only in the high-dose N treatment. The comparison with N:P ratios published by Koerselman & Meuleman (1996) for European wetland ecosystems shows that, even though the ecosystem type is quite different, the applicability is given. Koerselman & Meuleman (1996) stated N:P ratios below 14 to be indicative for N limitation; the N:P ratios determined in all our

treatments (except the high-dose N treatment in the last few years) were below 14, indicating N limitation. Phytomass likewise revealed limitation by N. P addition strengthens the N limitation according to the N:P ratios, as expected. The high-dose N treatment reached (at least in some years) a ratio in the range of N and P co-limitation (14-16) according to Koerselman & Meuleman (1996), but in our system additional P (NP, NPK, NPKM) had no stronger effect than N addition alone.

Effects of fertilization on nutrient concentrations and N:P ratios in roots were scarcely investigated. Overall, the N concentration in roots was higher than in above-ground phanerogams´ phytomass. By pooling into treatment groups, enhanced N concentrations in the N+ group were detected. Stevenson (1995) and Michelsen et al. (1999) determined also an increase of N in roots with fertilization of N or NPK, respectively. The changes in P concentration were consistent with that of above-ground phanerogam phytomass.

In cryptogams the increase of N or P concentrations by high-dose N or P addition was more pronounced than in phanerogams. Both bryophytes and lichens lack a well-developed cuticule, so that nutrients can be absorbed over the entire surface area. This can result in increased tissue N and N:P ratios by N addition (Pitcairn et al. 2006; Arroniz-Crespo et al. 2008) and increased tissue P and reduced N:P ratios by P addition in bryophytes (Arroniz-Crespo et al. 2008); at least in some lichens addition of N- and P-containing fertilizer induced an increase of N concentration, whereas P containing fertilizer increased only the P concentration (Vagts & Kinder 1999). For N, a dose-related increase of N concentration with higher tissue N could be observed in high- than in low-dose N treatment. Hence, saturation of N in bryophytes was not reached. Pearce et al. (2003) observed saturation in one bryophyte species already when adding N in low dose (10 kg ha^{-1} yr^{-1}). Niinemets & Kull (2005) described a lower N:P ratio of mosses than of vascular plants; in our study, the N:P ratio of cryptogams was not clearly different from that of phanerogams.

Litter had N and P concentrations in the range of above-ground phytomass of phanerogams, with a slightly higher N:P ratio. We would have expected lower concentrations than in above-ground phytomass of phanerogams, e.g. due to resorption of nutrients during senescence or nutrient losses through decomposition. At least for fresh litter, leaching of nutrients is highly variable and in trend not very high for N and P (summarized in Aerts & Chapin 2000). Another explanation could be an underestimation of the nutrient concentrations in above-ground phytomass of phanerogams provoked by the sampling date. Possibly nutrients in above-ground plant tissues were to some extent already resorbed when sampling took place in September.

5.5.4 Ellenberg indicator values

Ellenberg indicator values are a useful tool to indicate the ecological condition of the studied plots (Hill & Carey 1997). For instance, differences in community composition, e.g. caused by fertilization, can be reflected in EIVs. The high-dose N addition resulted in a significant increase in EIVs for nutrients and moisture, and a significant decline in EIV for light in the N+ group. Comparing the EIV for nutrients and plant-available N in soil, the trends fit quite well together; after 2004 both values increased in the high-dose N treatments. Thus, the Ellenberg N value is a good predictor for changes of site conditions due to nutrient additions in our study system. This is in contrast to Schaffers & Sykora (2000), who found only weak correlations of Ellenberg N-values with soil parameters, e.g. available mineral N.

The increase of the moisture value with N fertilization cannot be equated with increasing water content in soil; otherwise the F-value should have increased as well in the N– group. An explanation for this increase may be a phenomenon described by Ellenberg & Leuschner (2010): in parts N can replace water as a resource for plants. That is, the fertilization with N decreases the demand for water in matters of productivity.

The Ellenberg light value decreased under N fertilization, presumably because light-indicating plants of this early successional and open ecosystem were replaced by more competitive plants of later successional communities, e.g. *Elymus repens*, with lower light values. This signifies that the fertilization-caused changes in community composition were reproduced by the light value.

No significant changes were found for the soil-reaction value according to fertilization. Even though the soil-pH values inexplicably decreased on all treatments, the EIV for soil reaction remained stable or tended to increase slightly. Schaffers & Sykora (2000) also described the relationship of the soil-reaction EIV with soil pH as unsatisfactory.

Except for the soil-reaction value, the Ellenberg indicator values matched well with our soil data, exactly with the data on plant-available N, and vegetation data of our study site (Faust et al. 2012). A lag phase of five years for changes in community composition induced by fertilization of high-dose N (described by Faust et al. 2012) was displayed in the EIVs as well. Overall, at least some EIVs provide a good opportunity for predicting site conditions and their changes over time.

5.5.5 Successional pathways

In base-rich sand ecosystems the pioneer Koelerio-Corynephoretea vegetation is typically replaced by species-rich Allio-Stipetum stands during succession (Süss et al. 2010). Succession can be influenced by addition of fertilizers, triggering new shifts in trajectories (Walker & del Moral 2009). Faust et al. (2012) already described the separation in successional pathways in this study system following nutrient addition. After a lag phase of about four years the plots receiving high-dose N took a modified pathway from the supposed ´typical´ one. The community probably needs this lag phase to respond to altered nutrient conditions. Since then, the separation of the two pathways is in progress.

Nitrogen in low dose, simulating moderately increased atmospheric N deposition, was not shown to cause shifts in succession, which is in line with Wilson et al. (1995). In contrast, Clark & Tilman (2008) concluded that chronic low-level nitrogen deposition causes a decline in plant species richness. Neither P nor carbon had an effect on changing the successional pathway.

6 Synopsis

inoculation

donkeys on the restoration area ´Apfelbachdüne´

Armeria maritima subsp. *elongata*

The fragmented landscape of today´s Central Europe has induced major threats to many semi-natural open ecosystems. Isolation of remnant habitats is hampering the seed dispersal between these fragments, as well as cessation of traditional kinds of land use such as rotational grazing. The latter also has impact on successional pathways and community composition, as many open ecosystems depend on regular disturbance to persist. Additionally, excessive nutrient supply is a threat for nutrient-poor ecosystems. Restoring such ecosystems requires knowledge of the type of nutrient limitation and consequences of enhanced nutrient supply, adjusting the abiotic conditions of a restoration site to the demands of the desired community, assistance in (re-)colonization and finally a proper management regime to maintain the achievements. A number of these factors influencing the restoration success were studied (Fig. 6.1) and are regarded synoptically in this section.

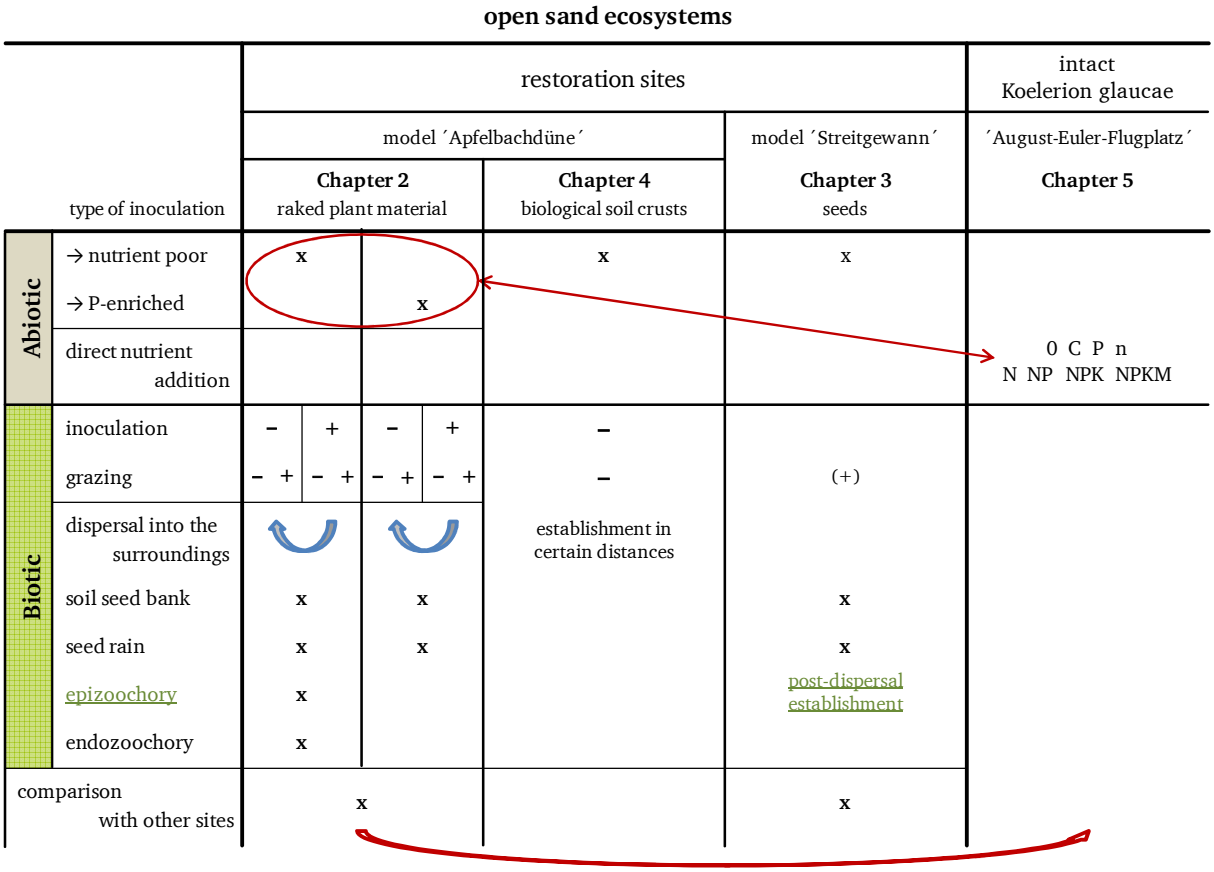


Fig. 6.1: Diagram of the chapters with the studied factors and interrelationships between the chapters. (+) = restorative grazing, but not subject of Chapter 3.

Abiotic difficulties

High nutrient concentrations in the soil, mostly due to former agricultural use, are known to minimize the success of restoration measures (Stroh et al. 2002). As an alternative for the current method of topsoil removal (Allison & Ausden 2004; Kiehl & Pfadenhauer 2007) the deposition of deep sand was employed to diminish nutrient availability in the restoration approach discussed in **Chapter 2**. The focus here concentrated on the impact of sand conditions. As was found by Wessels-de Wit & Schwabe (2010) and Eichberg et al. (2010), application of high-quality sand reduced concentrations of nitrogen and phosphorus to very low levels. Nitrogen was below values measured in our ecosystem (Süss et al. 2004) and P in the range of values measured in the intact sand ecosystem of **Chapter 5**. Deposition of sand of suboptimal conditions reduced N concentrations comparably, but caused P concentrations which were in some parts considerably higher than in the intact sand ecosystem. However, even with partly higher P concentrations, the sand deposition certainly resulted in lower total nutrients (including P) compared to the arable field soil underneath.

It could be shown that substrate conditions affected vegetation development. Species richness and cover of both vascular plant species and cryptogams were higher on the suboptimal sand. However, the impact of P cannot be confirmed without fail (at least so far) as an assumed contamination of the substrate with seeds due to above-ground storage presumably affected species richness and cover as well.

With respect to the direct nutrient addition experiment (**Chapter 5**), P addition did not reveal differences from the control in phytomass production, community composition or successional pathway. Instead, nitrogen was shown to be the limiting nutrient, influencing the successional pathway in this ecosystem. But to conclude that P addition has no effect on our studied system would be hasty, as this is in contrast to results obtained from other studies conducted in this area (Stroh et al. 2002; Süss et al. 2004). These studies revealed facilitation of competitive graminoids and adverse effects on a mid-successional target grass species, *Stipa capillata* (Süss et al. 2004) as well as development towards ruderal communities (Stroh et al. 2007) due to higher P concentrations (above approx. 20 mg P kg^{-1}; Süss et al. 2004). One explanation for this contrasting result of the intact sand ecosystem might be that this specific early successional community, which is rich in cryptogams and relatively poor in legumes, is responding in a different manner than, e.g., at first more or less cryptogam-free restoration sites or later successional stages to higher P concentrations. In relation to the restoration area ´Apfelbachdüne´ the high cover of the legume *Melilotus albus* on the P-richer site was

noticeable. Legumes can be favoured by low N (and higher P) concentrations in the soil, as they have the ability to fix atmospheric N by means of rhizobia (Sitte et al. 2002).

Even though no clear effect of P was determinable on the restoration site I would recommend paying attention in restoration projects to obtain high quality soil which was not temporarily stored above-ground. Otherwise contamination with nutrients and seeds cannot be excluded, which would be likely to affect the restoration success and management effort in the long run.

Restoration: Biotic constraints

Another critical point for restoration measures is the seed and dispersal limitation of many target species, i.e. the desired species are not able to reach the restoration sites. Thus, creating favourable environmental conditions without species introduction mostly did not lead to the re-establishment of target communities (see reviews of Bakker & Berendse 1999; Walker et al. 2004).

The soil seed bank cannot serve as a target species source in restoration approaches with deep sand deposition (**Chapter 2**). Even though the suboptimal sand contained a few target species´ seeds, the majority of species and seeds were non-target ones. As a result, the cover of non-target species was high on this substrate. In various restoration studies an initial ruderal vegetation stage was recorded (e.g. Jongepierová et al. 2007; Rydgren et al. 2010). In accordance with Eichberg et al. (2010) the high-quality sand contained a vanishingly small amount of (non-target) seeds. The intention of depositing deep sand is to receive a substrate that not only has a low nutrient level but is also poor in non-target species and seeds, to enhance the establishment success of desired species.

The seed rain on both restoration sites (**Chapters 2 and 3**) was likewise unable to assist target species introduction; primarily non-target species were transported by wind (cf. Stroh et al. 2002). On both sites species established in the surroundings dominated the aerial seed input, as observed by Auffret & Cousins (2011) and Faust et al. (2012). On the restoration area ´Apfelbachdüne´ (**Chapter 2**) very few target species were trapped, even though inoculated plots were only at least 12 m and the adjacent target community around 50 m away from the nearest traps. Diacon-Bolli et al. (2013) found similarly only small numbers of specialist species 1-40 m outside of calcareous grassland patches, and concluded that restoration through wind dispersal from adjacent intact communities might be very slow.

Species introduction was shown to be strongly dependent on the considered taxa. The introduction of vascular plant- and bryophyte-target species via raked plant material (**Chapter 2**) was successful in terms of species number and cover, as could be expected in view of other studies (Kiehl et al. 2006; Eichberg et al. 2010). The fine-scale inoculation created ´starter´ populations of target species, from which the species can spread to the whole restoration site. Around 70 % of all recorded target species could be detected outside the inoculated plots; however, distance from the next inoculated plot was not the crucial factor for the number of target species and affected target-species cover only slightly. In contrast, Burmeier et al. (2011) revealed that both number and abundance of transferred species tended to decrease with distance from plant material strips (maximum 10 m) 7-8 years after restoration realisation. However, I did not test for occurrence directly adjacent to the inoculated plots but some meters away on grid plots; in the nearest surroundings target species were found to spread and to achieve partly higher cover values. In any case, plots or strips of plant material or regional seed mixtures (Jongepierová et al. 2007) can provide an – even though slower – alternative to large-scale restoration measures, when donor sites are scarce or only limited funds are available.

For sand-specific bryophytes, the introduction via transplanted biological soil crusts is another feasible approach (**Chapter 4**). The transplanted bryophytes were able to establish and to spread onto the restoration site, even though distance to the transplants remained short within two years. The use of soil-crust transplants as a way to establish founder populations of bryophytes was already discussed by Bowler (1999). In contrast to bryophytes and vascular plants, the introduction of soil crust organisms via transplants had only minor effects on the establishment of cyanobacteria and eukaryotic algae. Dispersal does not seem to be a limiting factor for these soil-colonizing taxa; cyanobacteria and eukaryotic algae appeared independently of distance to the transplants via air, and the community composition did not strongly differ between transplants and the developing crusts. For these taxa dispersal via air is documented (Sharma et al. 2007).

To enhance spreading of vascular plant- and bryophyte-target species into the surroundings or to new suitable habitats the introduction of grazing livestock revealed promising results (**Chapters 2 and 3**). Although only a few species on the restoration area ´Apfelbachdüne´ (**Chapter 2**) were transported via epizoochory by donkeys, especially at the first sampling date in June a variety of target species was recorded. The other dates were mostly non-target species transported in the fur. The temporal variation in transported species is in line with the findings of Couvreur et al. (2004). Transport of mainly ubiquitous species was recorded by

Mouissie et al. (2005b), whereas in the study of Wessels et al. (2008) slightly more target than non-target species were detected in sheep fur. Time of grazing seems to be an important factor for dispersal of target species (epi- and endozoochorously), though it should be taken into account when planning the grazing management. Additionally, as stated by Wessels (2007), grazing should be directed from target species' rich source areas to sink areas with improvement potential to promote dispersal in the desired direction. As shown in **Chapter 3**, establishment following epizoochorous dispersal is possible, at least when gaps such as bare soil are provided.

In the context of vegetation development in comparison to other sites, the restoration area ´Apfelbachdüne´ (**Chapter 2**) revealed development in direction towards the donor site for inoculation material, whereupon the inoculated plots were arranged closer to the donor site than non-inoculated plots in the ordination diagram. Introduction of plant material can route vegetation development in direction of the donor sites, thus in direction of the desired community (Kirmer & Mahn 2001; Eichberg et al. 2010). The ´Streitgewann´ restoration site in **Chapter 3** was not very close to the nearby nature reserve but followed the development of the surrounding vegetation. As only selected species were introduced in this experiment via epizoochory, approximation to the surroundings was likely, as the neighbouring vegetation had a strong influence on development via seed input.

In conclusion, inoculation of only small parts of a restoration site using raked plant material may not create desired target communities in a short time period but has the potential to act as colonization initiator. Likewise, the transplantation of bryophyte-containing soil pieces can be a way to establish founder populations of bryophytes, whereas algal and cyanobacterial components of biological soil crusts obviously do not need assistance for establishment in our study system. Grazing livestock can promote the further spread of target species to the surroundings as well as between separated habitats via zoochory.

Management

Post-restoration management is crucial for semi-natural ecosystems such as sandy grasslands which require regular disturbance (Jentsch et al. 2002b). The introduction of grazing donkeys had so far mainly structural effects on the restoration site of **Chapter 2**. Trampling maintained a high proportion of open ground, especially on the nutrient-poor site, by preventing the development of a soil-crust and bryophyte layer. With regard to the inoculation this was counterproductive, as the transferred cryptogams were reduced severely

in cover. Especially the transferred – and by trampling heavily disturbed – moss *Tortula ruraliformis* was shown to have a positive effect on seedling recruitment (Eichberg et al. 2007), whereas germination rate-lowering pleurocarpous mosses were almost absent on the restoration site. In contrast to a variety of other studies (e.g. Stroh et al. 2007; Plassmann et al. 2010) no effects on vegetation composition, e.g. enhanced species diversity or reduction of ruderal species, could so far be observed on the restoration site.

The grazing period should be more closely matched to the demands of the particular site, i.e. on the nutrient-poor site by a reduced grazing pressure. Secondly, a mixed grazing regime might have been useful to achieve a stronger reduction of ruderal species, as different livestock species can complement each other in their diet selection (Loucougaray et al. 2004; Süss et al. 2009). In the long run grazing will presumably support the open structure of this restoration site and thus favour the occurrence of pioneer vegetation.

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Ehrenwörtliche Erklärung

Ich erkläre hiermit ehrenwörtlich, dass ich die vorliegende Arbeit entsprechend den Regeln guter wissenschaftlicher Praxis selbstständig und ohne unzulässige Hilfe Dritter angefertigt habe.

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