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Mgr. Jana Duchoslavová

Role of clonal integration in plant competition

Role klonální integrace v kompetici rostlin

Doctoral thesis

Supervisor: prof. RNDr. Tomáš Herben, CSc.

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Declaration / Prohlášení autora:

I hereby declare that I made this thesis independently, using only the mentioned references. I did not submit this thesis nor its part for any other degree or diploma.

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tuto práci ani její podstatnou část jsem nepředložila k získání jiného nebo stejného akademického titulu.

V Praze dne Jana Duchoslavová

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Abstract

Clonal growth enables plant to replicate rooting units (i.e. ramets), adding another hierarchical level to plant modularity. Clonal growth is very common, at least in the temperate flora, and widespread over the angiosperm phylogenetic tree. Different clonal growth forms have specific sets of clonal traits that determine their performance in response to environmental conditions and under competition. Clonal integration is one of the important clonal traits and is associated with a number of other clonal functions. Integrated ramets can translocate resources and signals by connecting clonal organs, such as horizontal stems or roots. Such integration is particularly important for young developing ramets, but it may persist and help developed ramets cope with environmental heterogeneity. Clonal integration at both early and later stages of ramet development may be an important factor affecting plant competition and has been shown to promote expansion into vegetated areas in some species. However, competition is complex and differs aboveground and belowground. Translocation of carbon and nutrients under competition may differ accordingly, but very little is known about this. The costs and benefits of clonal integration have been predicted by theoretical models. However, there is considerable variability in the observed benefits of clonal integration between species. This variability may be caused by different mechanisms of and requirements for translocation of different resources, or by different translocation strategies across species. As part of the plant economics, resource translocation may respond to a gradient of habitat productivity, showing conservative patterns where resource availability is low and competitive patterns where competition is high.

My aim here was to contribute to the understanding of the role of clonal integration in plant performance and how it relates to plant competition. Specifically, I asked (i) whether the benefits of resource translocation for the growth of a clonal plant match theoretical predictions, (ii) how resources are translocated under light heterogeneity simulating aboveground competition and how translocation changes during ramet ontogeny, (iii) whether patterns of resource translocation differ among species in predictable ways, and (iv) how clonal growth form affects species performance in communities.

To answer the first three questions, I used an experimental approach and investigated resource sharing under heterogeneous light and nutrients by growth and labelling experiments. In my first paper, I performed a growth experiment on a model clonal grass, *Agrostis stolonifera*, under different patterns of nutrient heterogeneity to test theoretical predictions of the benefits of resource sharing. I found that the benefits for daughter ramets were unexpectedly higher at higher levels of nutrient availability. In my second and third papers, I traced labelled carbon and nitrogen in both directions (i.e. acropetally and basipetally) and across multiple ontogenetic stages in *A. stolonifera* and two closely related Rosaceae species. I demonstrated the transition of resource sharing patterns through the plant ontogeny under light heterogeneity, and the presence of different translocation patterns in the species studied. I also formulated a conceptual model of possible translocation strategies in the third paper.

Subsequently, in my fourth paper, I focused on the interspecific comparison of nitrogen translocation in six stoloniferous species under nutrient heterogeneity to test the hypothesis that differences in resource sharing may be determined by the level of competition typically experienced by the species. The results did not confirm the hypothesis, but again showed the presence of distinct translocation patterns across species. In my last paper, I addressed the fourth question by analysing the performance of species with different clonal growth forms in plant communities using data from the long-term biodiversity experiment in Jena. The results suggested that species with different growth forms complement each other in their resource use strategies and that clonality thus affects mechanisms of plant coexistence.

My main contributions here are to the mechanistic understanding of carbon and nitrogen translocation between ramets, and to the understanding of the role of clonal integration in the context of plant communities and real-life interactions. My results confirmed the expected universal support of developing young ramets, which clearly form strong sinks for both carbon and nitrogen. Ramet relative size seemed to be a promising predictor of later nitrogen translocation pattern across species, which was surprisingly not affected by nutrient heterogeneity. In contrast, carbon translocation was driven by external availability in light, although different species differed in their willingness to send carbon to older ramets. I showed that differences between resources and possible adaptive strategies of resource translocation should be considered to better understand clonal integration and its role in competition.

Key words: clonal plants, physiological integration, nutrients, nitrogen, carbon, resource translocation, environmental heterogeneity, competition, stable isotopes, pulse labelling, stolons, rhizomes, ramets, clonal growth form

Abstrakt

Klonální růst umožňuje rostlinám replikovat kořenující jednotky (t.j. ramety), a přidává tak další hierarchickou úroveň k modularitě rostlinného těla. Klonální růst je velmi častý a vyskytuje se napříč celým fylogenetickým stromem krytosemenných rostlin. Jednotlivé formy klonálního růstu přinášejí rostlinám specifické sady klonálních vlastností, které určují jejich růst v různých podmínkách prostředí či v kompetici. Klonální integrace je jednou z důležitých klonálních vlastností související s řadou dalších funkcí klonality. Integrované ramety mohou translokovat zdroje a signální molekuly pomocí klonálních orgánů, jako jsou šlahouny, oddenky nebo kořeny. Integrace je zvláště důležitá pro mladé, vyvíjející se ramety, ale může přetrvat a vyrovnávat heterogenitu podmínek mezi vyvinutými rametami. Jak v raných, tak v pozdějších fázích vývoje může být klonální integrace důležitá v rostlinné kompetici. Experimentální studie ukázaly, že klonální integrace u některých druhů skutečně pomáhá jejich šíření do zápoje sousedních rostlin. Kompetice nad zemí a pod zemí se ale liší, což může mít vliv na translokaci uhlíku a živin. O tomto tématu se nicméně ví velmi málo. Výhody a nevýhody klonální integrace v různých podmínkách byly předpovídány pomocí teoretických modelů. Pozorované výhody klonální integrace se ale ukazují být různé pro různé druhy. Tato variabilita může být způsobena různými mechanismy translokace jednotlivých zdrojů a různými požadavky na tyto zdroje, nebo také různými strategiemi sdílení zdrojů u různých druhů. Sdílení zdrojů mezi rametami je možná součástí rostlinné ekonomiky, a může tak vykazovat konzervativní strategii u rostlin z prostředí s nízkou dostupností zdrojů, nebo kompetiční strategii u rostlin z bohatého a kompetičního prostředí.

Cílem mé práce bylo přispět k porozumění toho, jakou roli má klonální integrace pro růst rostlin a rostlinnou kompetici. Ptala jsem se (i) zda výhody klonální integrace u modelové rostliny odpovídají předpovědím teoretického modelu, (ii) jak jsou zdroje translokovány při heterogenním osvětlení simulujícím nadzemní kompetici a jak se tato translokace mění v průběhu vývoje, (iii) zda jsou rozdíly v translokaci mezi druhy predikovatelné a (iv) jak forma klonálního růstu ovlivňuje růst druhů v rostlinných společenstvech.

Pro zodpovězení prvních tří otázek jsem použila růstové a značící experimenty, ve kterých jsem zkoumala sdílení zdrojů mezi rametami při heterogenním osvětlení a dostupnosti živin. Ve své první studii jsem pomocí růstového experimentu na klonální trávě *Agrostis stolonifera* chtěla ověřit předpovědi teoretického modelu ohledně výhod sdílení živin při různých podobách heterogenity. Předpověď modelu se nepotvrdila, protože výhody integrace byly nečekaně vyšší při vyšší dostupnosti živin. Ve své druhé a třetí studii jsem sledovala pohyb značeného uhlíku a dusíku v obou směrech a napříč vývojovými fázemi u *A. stolonifera* a dále u dvou blízce příbuzných druhů z čeledi Rosaceae. Ukázala jsem, jak se mění translokace zdrojů v průběhu vývoje rostliny při heterogenním osvětlení a že studované druhy mají různé vzorce translokace. Ve třetí studii jsem také navrhla klasifikaci možných translokačních strategií. Ve své čtvrté studii jsem se následně zaměřila na srovnání

translokace dusíku u šesti výběžkatých druhů při heterogenní dostupnosti živin. Mým cílem bylo ověřit hypotézu, že rozdíly v translokaci dusíku mohou být dány úrovní kompetice, na kterou jsou tyto druhy přizpůsobené. Výsledky moji hypotézu nepotvrdily, ale opět ukázaly přítomnost odlišných způsobů translokace mezi druhy. Ve své poslední studii jsem se zabývala vlivem formy klonálního růstu na růst rostlinných druhů ve společenstvu. Za tímto účelem jsem analyzovala data z dlouhodobého biodiverzitního experimentu v Jeně. Výsledky naznačily, že druhy s různými formami klonálního růstu se vzájemně doplňují ve svých strategiích jak využívat zdroje a že klonalita ovlivňuje mechanismy soužití různých rostlinných druhů.

Tato práce přispívá jednak k mechanistickému porozumění translokace uhlíku a dusíku mezi rametami klonálních rostlin a jednak k porozumění role klonální integrace v dlouhodobém soužití rostlin v rostlinných společenstvech. Moje výsledky potvrdily očekávanou podporu vyvíjejících se ramet, které tvoří silné sinky pro uhlík i dusík, skrze klonální integraci. Jako slibný ukazatel míry translokace dusíku se ukázala relativní velikost ramet, kdežto heterogenita v dostupnosti živin překvapivě neměla na translokaci dusíku vliv. Translokace uhlíku byla naproti tomu ovlivněná dostupností světla, ačkoliv různé druhy se ukázaly různě ochotné posílat uhlík starším rametám. Moje práce ukázala, že pro lepší porozumění klonální integraci a její roli v kompetici bychom měli brát v potaz jak rozdíly mezi různými zdroji, tak možné adaptivní strategie sdílení zdrojů mezi rametami klonálních rostlin.

Klíčová slova: klonální rostliny, fyziologická integrace, živiny, dusík, uhlík, translokace živin, heterogenita prostředí, kompetice, stabilní izotopy, pulzní značení, šlahouny, oddenky, ramety, klonální růstová forma

Contents

Author contribution statement

The presented thesis consists of general introduction and discussion, three published papers and two manuscripts. Four papers are co-authored. Author contributions to individual paper are as follows.

Paper I Evidence for unexpected higher benefits of clonal integration in nutrient-rich conditions.
Duchoslavová Jana, Weiser Martin (2017). *Folia Geobotanica*, 52: 283-294. doi: 10.1007/s12224-016-9274-8

JD and MW planned the experimental design, JD conducted the experiment, analysed the data and wrote the manuscript. MW advised on the data analyses. Both authors contributed critically to the drafts and gave final approval for publication.

Paper II The direction of carbon and nitrogen fluxes between ramets in *Agrostis stolonifera* changes during ontogeny under simulated competition for light.
Duchoslavová Jana, Jansa Jan (2018). *Journal of Experimental Botany*, 69: 2149-2158. doi: 10.1093/jxb/ery068

JD and JJ developed the labelling methodology, JD planned the experimental design, conducted the experiment, analysed the data and wrote the manuscript. JJ advised on the experimental design and provided the elemental and isotopic analyses. Both authors contributed critically to the drafts and gave final approval for publication.

Paper III Strategies of resource sharing in clonal plants: A conceptual model and an example of contrasting strategies in two closely related species.
Duchoslavová Jana, Jansa Jan. Manuscript under review in *Annals of Botany*.

JD planned the experimental design, conducted the experiment, analysed the data and wrote the manuscript. JJ advised on the experimental design and provided the elemental and isotopic analyses. Both authors contributed critically to the drafts and gave final approval for publication.

Paper IV Nitrogen sharing strategies in six clonal species.
Duchoslavová Jana. Manuscript.

Paper V Effect of clonal growth form on the relative performance of species in experimental communities over time.
Duchoslavová Jana, Herben Tomáš (2020). *Perspectives in Plant Ecology, Evolution and Systematics*, 44: 125532. doi: 10.1016/j.ppees.2020.125532

JD led the communication to obtain the data, analysed the data and wrote the manuscript. TH advised on the data analyses. Both authors contributed critically to the drafts and gave final approval for publication.

Introduction

Clonal growth

Plants are modular organisms, repeating the basic architectural units in their construction plans, and this modularity allows a high degree of flexibility in plant bodies. Clonal growth adds another hierarchical level to the plant modularity by replicating ramets, i.e. "rooting units", each consisting of roots connected to a shoot (Ottaviani *et al.* 2017; Oborny 2019). Ramets are produced by various clonal organs, mainly of stem or root origin (see Fig. 1 for examples of major types of clonal organs). Clonal growth is very common, at least in a temperate flora – clonal organs are found in more than 60% of herbaceous perennial species in Central Europe (Klimešová *et al.* 2017; Herben and Klimešová 2020). Similar estimates for other floras are not possible due to lack of data.

The major types of clonal growth organs found in Central Europe are distributed over the whole phylogenetic tree of angiosperms and, according to the phylogenetic reconstruction, were present since their early evolution (Hutchings and Mogie 1990; Xue *et al.* 2016). However, plants can readily switch between different clonal organ types and between clonal and nonclonal habit during evolution (Herben and Klimešová 2020). Therefore, clonal growth is not a universally favoured or disfavoured trait, as it has been lost and reinvented many times over the course of evolution.

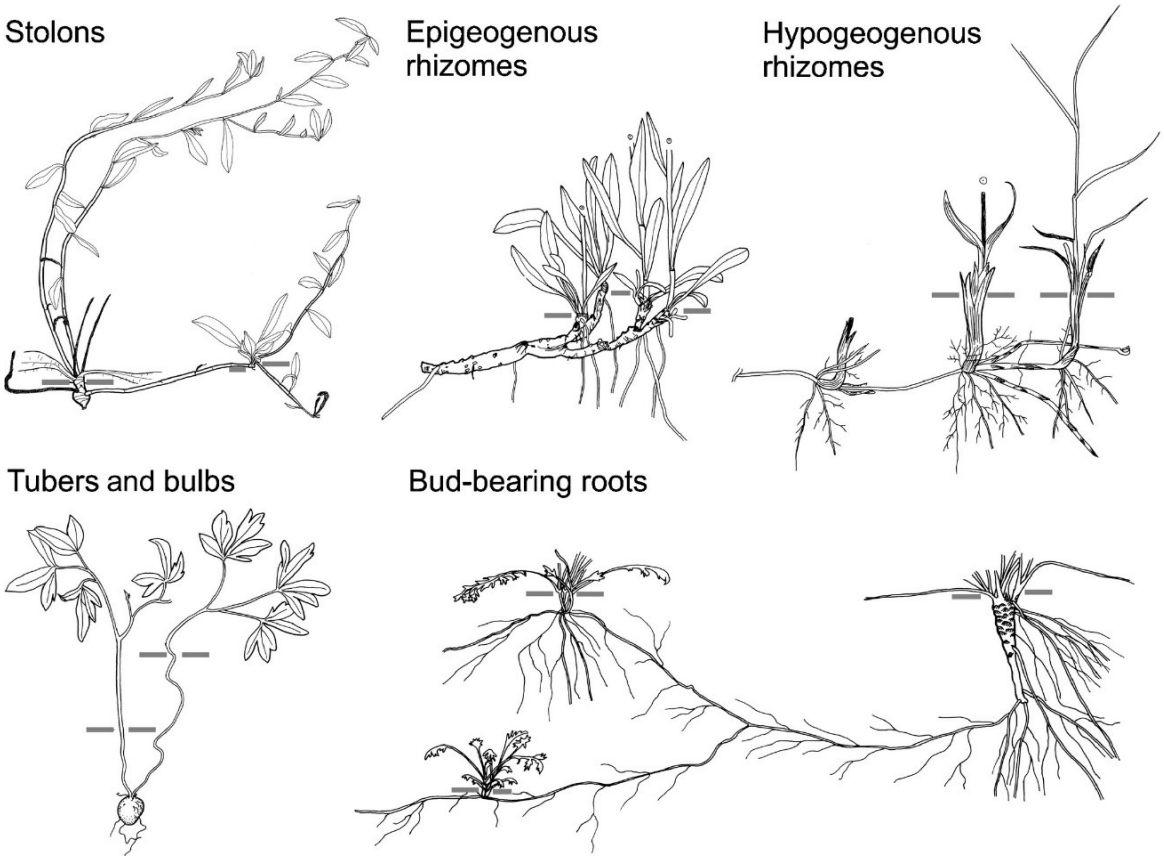

Figure 1 Examples of major types of clonal organs. From Herben and Klimešová (2020).

Clonal growth provides the plant with specific functions such as vegetative multiplication, bud bank deposition and lateral spread, which allows plants to 'move' horizontally, growing on one side and decaying on the other (Klimešová *et al.* 2017; Ott *et al.* 2019). In addition, clonal organs may also have important functions which are not unique to clonal plants, such as carbohydrate storage. On the other hand, clonal organs may be costly to build, and possibly also to maintain. For example, it has been shown that investment to clonal growth may decrease plant performance in early development (Martínková *et al.* 2020). The functions connected with clonality can shape essential plant processes and contribute markedly to plant ecological niches (Klimešová *et al.* 2016, 2021; Chelli *et al.* 2024). Clonal traits reflecting these functions are strongly constrained by the type of clonal organ, i.e. the clonal growth form (Herben and Klimešová 2020).

Nowadays, occurrence of different clonal growth forms is determined by environmental conditions. For example, in Central Europe, stoloniferous species prefer frequently (but mildly) disturbed habitats with high availability of nutrients, while species with hypogeogenous rhizomes prefer moist habitats with lower temperatures (Sosnová *et al.* 2010; Klimešová and Herben 2024). Disturbance severity and frequency together with light and moisture are the strongest determinants of clonal growth forms occurrence in Central Europe (Klimešová and Herben 2024).

Accordingly, the different functions of clonal organs may be important under different environmental regimes (Klimešová and Herben 2015). In harsh habitats with low resource availability, reproductive insurance and conservation of nutrients may be the main benefit of clonal growth (Jonsdottir and Watson 1997; Klimešová and Doležal 2011). Under frequent disturbances, regeneration from the bud bank may be important (Martínková *et al.* 2020). When vegetation is heterogeneous, colonisation of vegetation gaps and horizontal foraging for light may become a relevant strategy, especially for relatively low species (Kalamees and Zobel 2002; Macek and Lepš 2003; Vítová *et al.* 2017). In stable productive and competitive habitats, space occupancy and vegetative multiplication may become important (Pitelka and Ashmun 1985; Gough *et al.* 2012).

Clonal integration of ramets is an important clonal trait related to the number of these functions, as it allows resource support of clonal offspring, regenerating ramets or resource-limited ramets. It is not included in the clonal trait database (Klimešová *et al.* 2017) due to the difficulty of measuring it and can therefore only be inferred from the presence or persistence of clonal organs connecting ramets in most of species. Clonal integration and its relationship to plant competition is the focus of this thesis.

Clonal integration

Clonal organs, especially horizontal stems or roots, allow ramets to share water, mineral nutrients, and photosynthates and to transport signal molecules (Pitelka & Ashmun, 1985; Marshall, 1990; Alpert et al., 2002). Such resource translocation among ramets and its regulation functions similarly to that within a nonclonal plant or a ramet (Hay and Kelly 2008). However, the presence of multiple root-shoot connections within an integrated plant modifies the rules. In contrast to integration of roots and shoot within a single ramet, the integration of different ramets is possible, but not obligatory, and the ramets are potentially able to survive independently. Clonal growth is diverse and the level of integration between ramets may vary widely from possibly full integration to total ramet separation (Sosnová *et al.* 2010). Moreover, the level of integration for different resources may differ in a clonal plant (Tietema and van der Aa 1981). Therefore, a clonal plant with multiple connected ramets is positioned somewhere between a fully integrated horizontally growing plant and genetically identical, but independently growing multiple plant units (Hutchings and Bradbury 1986). The uncertainty about level of integration between ramets of different clonal species and its effect on plant growth has raised questions in clonal plant ecology for decades (see Pitelka and Ashmun 1985; Marshall 1990; Song *et al.* 2013; Liu *et al.* 2016; J Wang *et al.* 2021 for some reviews).

Theoretical predictions of resource translocation

Generally, three main factors may determine the direction and magnitude of resource translocation – ramet relative age (i.e. translocation to younger or older ramets), ramet uptake capacity and resource availability experienced by the ramets. Benefits of resource sharing can be predicted by a simple theoretical framework considering translocation of a resource in a pair of ramets (Caraco and Kelly 1991; Alpert 1999; Dong *et al.* 2015). Benefits and costs of resource sharing for these ramets are affected by a relationship between internal resource level and ramet growth as well as by a position of both ramets on the resource level gradient (Fig. 2). Growth of individual ramets in response to resource level can be expected to rise steeply in low resource level and level off at maximum in high resource level, when another resource becomes limiting. Considering such type of relationship, both benefits of import of a given amount of resource and costs of the resource export for a ramet growth decrease with higher resource level. Therefore, net benefits of clonal integration can be expected to increase with higher resource level of the donor and lower resource level of the recipient ramet. However, the assumption of the growth independent of ramet size is necessary to easily obtain a net benefit of clonal integration by summing the growth rates of the ramets (but see Caraco and Kelly 1991). Another hidden assumption is that plants aim to maximize their biomass regardless on its allocation. In this conceptual model, the internal resource level is determined by combination of an external resource availability, uptake capacity of the ramet and resource translocation between ramets (Caraco and Kelly 1991; Dong *et al.* 2015).

Based on these predictions, the ramet saturated by the resource pays little cost for export of the surplus resource whereas any resource support is translated into enhanced growth in the resource-limited recipient ramet. Therefore, translocation should be especially beneficial in the beginning of daughter ramet development, when differences in uptake capacity of ramets are large, and under heterogenous resource availability. Accordingly, clonal integration has been shown to generally increase biomass of clonal plants in both cases, i.e. in pairs of developmentally different ramets under homogeneous conditions and in pairs of ramets grown under heterogeneous conditions (J Wang *et al.* 2021). However, the model presented makes some simplifying assumptions and further testing is needed to verify its prediction for different resource types and distributions and different sizes of mother and daughter ramets.

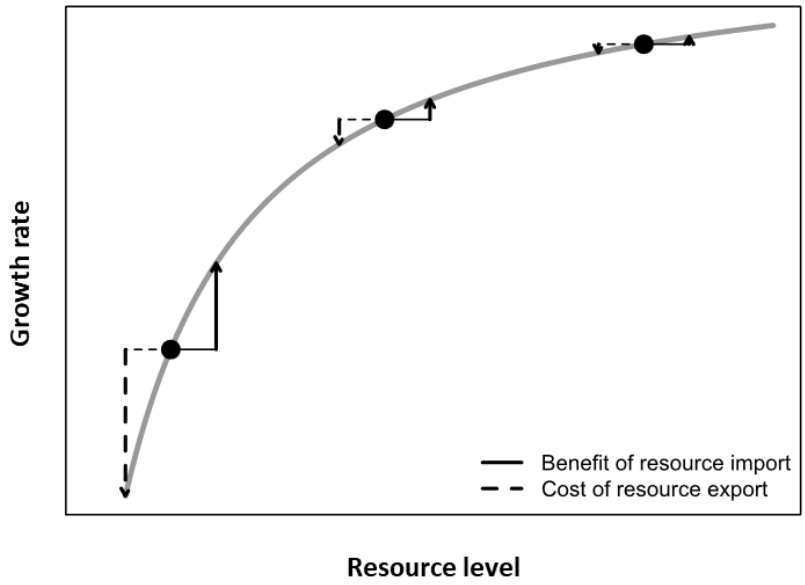

Figure 2 *Theoretical predictions of the benefits and costs of resource translocation. Both the benefits of importing of a given amount of the resource and costs of the resource export for a ramet's growth decrease as the resource level increases. The resource level can be altered by external resource availability or by uptake capacity of a ramet.*

Experimental methods for studying resource translocation

There are two main approaches to study clonal integration experimentally. First, translocation of a given resource can be observed directly by tracing of labelled substances (Jonsdottir and Watson 1997). Second, effect of integration on plant growth, morphological or physiological parameters may be examined (Slade and Hutchings 1987). Both approaches have their advantages and limits.

For simplicity and logistical reasons, pairs of ramets or clonal fragments divided to basal and apical parts have been used in a vast majority of experiments. Only few studies have used systems with three or more ramets (e.g. Alpert 1996; Janeček et al. 2007; Wolfer and Straile 2012). These studies

suggested that the overall translocation pattern is not easily predictable from the pairs-of-ramet approach. It is necessary to keep this in mind, as there are usually multiple ramet systems in natural conditions.

Labelling approach

The labelling approach allows us to tell whether translocation of a given resource is taking place and how is the labelled substance distributed within a plant. For the labelling, radioactive or rare stable isotopes of elements, or dyes can be used. Translocation of photosynthates can be traced by labelling with radioactive ^{14}C (or ^{11}C; Welker and Briske 1992) or stable ^{13}C (Luo *et al.* 2014), translocation of nitrogen by stable ^{15}N and translocation of phosphorus by radioactive isotopes (^{32}P and ^{33}P; Nannipieri *et al.* 2011). Translocation of water can be examined either by labelling by deuterium (de Kroon *et al.* 1996) or qualitatively by movement of dyes (DeByle 1964). The labelled resource is usually applied to the plant in a pulse lasting up to a few hours (but see de Kroon *et al.* 1996 for continuous labelling by deuterium).

The translocation in a clonal fragment can only be followed in one direction using the pulse-labelling approach. This means that for a given ramet or plant part, either export or import of the label can be studied. Consequently, many studies have observed translocation in only one examined direction, and few studies have examined the transport of labelled resources in both directions between the parent and daughter ramets using different clonal fragments.

Growth experiments

In growth experiments, effect of clonal integration on ramet performance, morphological or physiological parameters is examined. Integrated ramets are compared with ramets in the same conditions but with the connections between them severed, or integrated ramets in heterogeneous and homogeneous conditions are compared (Song *et al.* 2013; J Wang *et al.* 2021). Because of possible stress caused by severing and possible positive effect of clonal integration in homogeneous conditions, the first method is considered to overestimate the effect of integration, whereas the second method may underestimate it. However, there are no marked differences between results of the two approaches according to the recent meta-analysis (J Wang *et al.* 2021).

In an experimental ramet pair, the ramets are either siblings connected by a common mother ramet (usually leafless; Abrahamson *et al.* 1991), or they are connected in series (Friedman and Alpert 1991). Studies focusing on the effect of heterogeneity on clonal integration have often standardised the size of the ramets within a pair (Friedman and Alpert 1991). This treatment avoids confounding effects of differential ramet uptake capacity and environmental heterogeneity, but the clonal fragments are more artificial. Other studies use mother and daughter ramets or basal and apical parts of a clonal fragment (Alpert 1991; de Kroon *et al.* 1996; Xu *et al.* 2010). These studies therefore examine the effect of

different developmental stages and uptake capacities of ramets together with the effect of environmental heterogeneity.

While experiments assessing the effects of clonal integration on ramet performance provide a valuable indication of translocation patterns, they only indirectly show the translocation of specific resources. Moreover, they usually do not separate the effects of translocation at early and late developmental stages, which may be completely reversed (but see e.g. Xu *et al.* 2012; Ma *et al.* 2021 using repeated measurements of ramet growth).

Only a few studies have combined labelling of translocated elements with analysis of the effect of integration on ramet growth (Jonsdottir and Callaghan 1989; D'Hertefeldt and Jonsdottir 1994; Alpert 1996; de Kroon *et al.* 1996; Saitoh *et al.* 2006; Xu *et al.* 2010). Some of these studies found no or mixed link between translocation pattern and ramet performance (de Kroon *et al.* 1996; Saitoh *et al.* 2006). However, for example, a study on two Australian invasive species showed a good agreement between the two types of results – daughter ramets with more photosynthate support showed greater growth benefits from integration (Xu *et al.* 2010).

Resource translocation under homogenous conditions

Under homogeneous environmental conditions, resource translocation is determined only by internal gradients, i.e. by differences in uptake and use of resources in ramets, affected by their size and developmental stage (Fig. 3A). Translocation in natural clonal fragments under homogenous conditions is typically mainly acropetal (Alpert 1996; Jonsdottir and Watson 1997; Xi *et al.* 2019). Newly developing daughter ramets are supported by mother ramets because their resource demands are not covered by their limited resource uptake capacity (Marshall 1990; Alpert 1996). This support is analogous to maternal provision to seeds in sexual reproduction (Hartnett and Bazzaz 1983; Bullock *et al.* 1994; Wijesinghe 1994). The acropetal translocation may also reflect the tendency of plants to grow apically, controlled by hormonal signals (Alpert *et al.* 2002; P Wang *et al.* 2021). In natural clonal fragments, translocation to youngest ramets and growing rhizome or stolon tips was demonstrated in several species by labelling studies both for carbon and nitrogen (Tietema 1980; Noble and Marshall 1983; D'Hertefeldt and Jonsdottir 1994; Alpert 1996; Jonsdottir and Watson 1997). Therefore, support of young ramets seems to be universal, although they are able to survive when experimentally fragmented (Jonsdottir and Watson 1997).

The initial maternal support may change later in ramet ontogeny depending on resource availability in the environment (Pitelka and Ashmun 1985; Ma *et al.* 2021). In homogeneous habitats, directional resource translocation among developed ramets may stop (Colvill and Marshall 1981; de Kroon *et al.* 1996). However, the connection between ramets often remains physiologically functional until it withers, as persistent translocation of small amounts of resources has been observed in several species (Pitelka and Ashmun 1985; de Kroon *et al.* 1996).

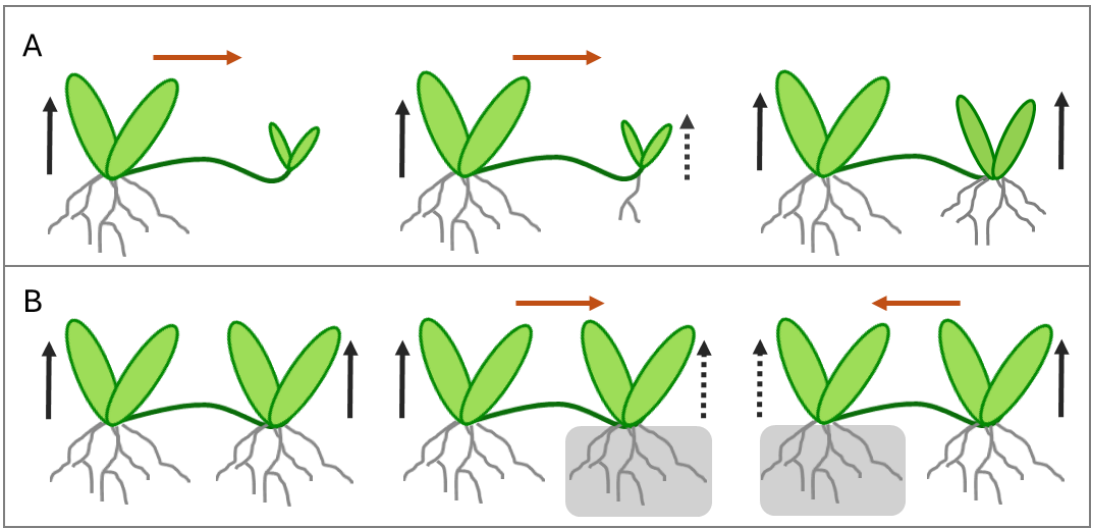

Figure 3 *Scheme of assumed translocation (A) during ramet ontogeny, and (B) between developed ramets under environmental heterogeneity. An example of a stoloniferous plant and translocated soil-borne nutrients. Arrows indicate direction of nutrient transport, translocation between ramets is highlighted in orange. Grey zones around roots indicate low nutrient availability.*

Resource translocation under heterogeneous environmental conditions

Persistent clonal integration between developed ramets may be advantageous when resources are distributed heterogeneously in space or time (e.g. Evans 1991; Alpert 1999; J Wang *et al.* 2021). In such a case, external gradients in resource availability cause internal gradients in resource levels and needs of ramets, which are presumably compensated for by resource translocation (Fig. 3B). Clonal integration can be enhanced also by enhanced resource needs of some ramets, for example due to flowering or fruiting, or stress (Salzman and Parker 1985; Jonsdottir and Callaghan 1989).

Effect of clonal integration on performance of plants grown under heterogeneous conditions was examined by number of studies on many clonal species (reviewed by Song *et al.* 2013; J Wang *et al.* 2021). Positive effects of integration between ramets on plant performance were demonstrated for heterogenous availability of water (Pennings and Callaway 2000), nutrients (Alpert 1991, 1996; Birch and Hutchings 1994), and light (Stuefer *et al.* 1994). However, despite the universal general conclusions of meta-analyses and theoretical predictions (Caraco and Kelly 1991; J Wang *et al.* 2021), the effects of integration on plant performance and the levels of translocation observed vary between the species and resources studied (de Kroon *et al.* 1998; Xu *et al.* 2010; Luo *et al.* 2014).

Uptake, transport and regulation mechanisms differ for water, soil nutrients and photosynthates and it is important to consider them to better understand the principles of resource translocation in clonal plants. Mineral nutrients and water are acquired predominantly by roots from soil, whereas carbon is gained by leaves from air via photosynthesis. The resources are subsequently transported by vascular tissues from the site of uptake to the sites of use or storage. Transport by xylem within a ramet is acropetal and driven by transpiration flow of water. In contrast, transport by living cells of phloem is

bidirectional and driven by source-sink relations. However, these two transport pathways are not independent of each other and lateral transfer of substances between xylem and phloem occurs (Tegeder and Hammes 2018). This allows, for example, the acropetal transport of nutrients from roots to shoots through the xylem and their basipetal transport to the roots at the same time.

In the next sections, I will summarise the translocation of water, nutrients and photosynthates separately and briefly describe the physiology of their uptake and transport.

Water

Water transport occurs primarily through the xylem. Whereas the transpiration flow is directed from roots to leaves within a single ramet, the direction of water transpiration flow between ramets is not necessarily acropetal, but is determined by differences in water potential between ramets (Qureshi and Spanner 1971; Tietema and van der Aa 1981; Alpert and Mooney 1986; Alpert 1990). The water gradient driving the translocation of water between ramets can be affected not only by differences in water availability to roots (Qureshi and Spanner 1971; Zhang *et al.* 2008), but also by water loss from shoots. For example, heterogeneous shading may affect the water potential of ramets within a clonal fragment and water may be translocated from shaded to unshaded conditions due to higher evapotranspiration under unshaded conditions (Lau and Young 1988; Stuefer *et al.* 1994). Moreover, ramets with a relatively higher leaf area may have a lower water potential than the other ramets and import water by clonal integration, even though their water supply is the same (Tietema and van der Aa 1981). Regulation of water translocation between ramets by a clonal plant can thus be achieved by regulation of water uptake and transpiration rate on a short-time scale or by regulation of ramet relative root and leaf area on a long-time scale.

Unlike photosynthate or nutrient translocation, water translocation can fully compensate for differences in water availability, resulting in equal or very similar performance of unlimited and limited ramets (Alpert and Mooney 1986; de Kroon *et al.* 1996; Lechuga-Lago *et al.* 2016) and water translocation seems to be more apparent than nutrient translocation in some species (e.g. Dong and Alaten 1999). In *Populus tremuloides,* translocation of water to distance up to 14 m has been demonstrated (DeByle 1964).

Nutrients

Nutrient translocation under heterogenous conditions has been studied either for multiple nutrient combinations or specifically for nitrogen, rarely also for phosphorus (Lotscher and Hay 1997; Wan *et al.* 2017). Here I will focus mainly on nitrogen as the main representant of mineral nutrients.

Nitrogen is taken up predominantly in form of nitrate or ammonium ions. It is then assimilated into amino acids, either in roots or shoots. Proportion of root and shoot nitrate assimilation varies across species and changes with nitrogen supply and other environmental conditions (Andrews and Raven

2022). Both inorganic forms of nitrogen taken up by the roots and amino acids assimilated there are transported by the xylem to the shoots. On the other hand, transport of organic nitrogen from leaves to sinks, such as growing roots, occurs primarily by phloem, where it is highly mobile (Tegeder and Hammes 2018). Nitrogen uptake and distribution in plants is under control of complex regulatory mechanisms, acting both on local and whole-plant scale (Gastal and Lemaire 2002; de Kroon *et al.* 2009; Tegeder and Masclaux-Daubresse 2018).

In clonal plants, nitrogen taken up by the roots of one ramet and loaded into the xylem may be transported by the transpiration stream to the leaves of the same or another ramet. In leaves it is either used or loaded into the phloem and transported to other sinks, following the source-sink principle. Therefore, the direction of the water transpiration flow in xylem between ramets may, at least partly, affect translocation of mineral nutrients. Indeed, translocation of nitrogen between ramets has been shown to depend on nitrogen availability gradient together with the transpiration flux manipulated by differential water availability (Evans 1991; de Kroon *et al.* 1998).

Regarding the role of environmental nutrient heterogeneity, translocation has been shown to act in the expected equalising manner in some studies, with nutrients moving from ramet in nutrient rich to ramet in nutrient poor conditions (Evans 1988; Wang *et al.* 2017). However, no apparent effect of nutrient translocation was observed in other studies with respect to ramet final biomass or morphological responses (Friedman and Alpert 1991; Dong and Alaten 1999; Liao *et al.* 2003).

In several cases, such effect of translocation was observed only in the acropetal direction, i.e. towards younger parts of a plant (Noble and Marshall 1983; Slade and Hutchings 1987; Wijesinghe and Handel 1994; Portela *et al.* 2021). Generally, the acropetal direction of translocation seems to be the "default" configuration of the plant with nutrients translocated from older parts of clonal fragments to strong sinks in rhizome or stolon growing tips and thus being allocated to clonal spread (D'Hertefeldt *et al.* 2011). The acropetal nutrient translocation pattern is favoured by a higher allocation to roots in older ramets in some species, or even by the loss of shoots in older ramets (Jonsdottir and Callaghan 1990; Roiloa 2019). In some species, the opposite direction may not be inducible by external heterogeneity. *Glechoma hederacea* is the classic example (Slade and Hutchings 1987), but it also seems true for *Carex arenaria* (Noble and Marshall 1983) or *Carpobrotus edulis* (Portela *et al.* 2021). In other species, translocation of nutrients was observed in both directions (*Hydrocotyle bonariensis,* Evans 1988*;* 10 invasive species, Wang *et al.* 2017) or from daughter to mother ramets (*Populus tremuloides,* Pinno and Wilson 2014). Translocation of nutrients in both directions at least in small amounts has been showed in several species by labelling approach (Alpert 1996; Jonsdottir and Watson 1997).

In some cases, the opposite effect of nutrient heterogeneity has been described. *Carex arenaria* increased production locally in nutrient rich patches (Noble and Marshall 1983; D'Hertefeldt *et al.* 2011). Tietema and van der Aa (1981) suggested that increased growth of a ramet in nutrient-rich

patches increased its transpiration and directed transpiration flow towards that ramet, resulting in import rather than export of nutrients by the ramet in the nutrient-rich patch. Similar mechanism could explain the results of Alpert (1996), where parental ramets of *Fragaria chiloensis* tended to be smaller and had lower nitrogen concentration when one of their daughter ramets grew in a nutrient-rich patch. This "rich get richer" effect was suggested also by Sun *et al.* (2011) in *Bouteloua dactyloides* (buffalograss) and might possibly explain also competition for resources in sibling ramets of *Solidago altissima* (Abrahamson *et al.* 1991). These cases suggest that relative size of ramets might be important for directionality of nutrient translocation.

Carbon

Carbon, assimilated to photosynthates in leaves or mobilised from storage organs, is loaded to the phloem, and transported through plants according to a source-sink mechanism. Its translocation may be further modified by hormonal control (Alpert *et al.* 2002; Novoplansky 2003). Therefore, it is presumably not much dependent on prevailing direction of transpiration flow and may respond to internal gradients readily. Imbalance induced by shading or defoliation has been shown to affect carbon translocation between ramets in a number of plant species (e.g. Qureshi and Spanner 1973; Pitelka and Ashmun 1985; Xu *et al.* 2010), including basipetal carbon translocation (Magda *et al.* 1988; Liao *et al.* 2003; Wang *et al.* 2017).

However, level of integration for photosynthates varies among species (Pitelka and Ashmun 1985; Hellström *et al.* 2006; Xu *et al.* 2010) and compensation for limited light availability or damage is not always the case (Hellström *et al.* 2006; Wolfer and Straile 2012). Competition for photosynthates between ramets (Hellström *et al.* 2006) or remobilisation and export of resources from shaded ramets (Ong and Marshall 1979; Wolfer and Straile 2012) have been reported. The translocation of carbon between ramets is analogous to the situation between branches of a nonclonal plant (Novoplansky 2003; Kawamura 2010), where both competitive and cooperative responses have been proposed (Kawamura 2010).

Similarly to nutrient translocation, only acropetal carbon translocation was observed in some species in response to shading (van Kleunen and Stuefer 1999) or defoliation (Noble and Marshall 1983) and it was suggested that storage carbon instead of carbon imported from younger parts may be used in older ramets to compensate for the local imbalance.

Resource interactions

Water, nutrients and carbon are part of a single plant economics, and they are obviously not independent of each other. For example, imported carbon might not be translated to markedly higher growth of a ramet limited by nitrogen, whereas it may increase growth when nitrogen is non-limiting

(Friedman and Alpert 1991). Moreover, the supply of shaded ramets with photosynthates can increase their ability to assimilate nitrogen from the soil (Chen *et al.* 2015).

Translocation of mineral nutrients in response to light gradients affecting photosynthate assimilation has hardly ever been studied. However, differential availability of photosynthates can lead to unbalanced nutrient requirements in different ramets, even though nutrients may be homogeneously distributed in the substrate. Indeed, the results of Saitoh *et al.* (2006) indicate that nitrogen translocation from shaded to unshaded ramets could be enhanced due to higher sink activity of developing unshaded leaves.

Information on translocation of different resources is available for very few species. Some studies have looked at the translocation of several different mineral nutrients (Noble & Marshall 1983) or mineral nutrients and water (de Kroon et al. 1998), but comparisons of translocation of belowground and above-ground resources for the same species under the same conditions are very rare and often come from different experimental setups (Jonsdottir and Watson 1997).

Integrated regulation

Integration of ramets in clonal plants inevitably involves integrated regulation, which is important, for example, to avoid self-competition (Holzapfel and Alpert 2003; Gruntman *et al.* 2004). Further, plants generally respond to resource limitation by adjusting their biomass allocation, morphology, physiology, and architecture, which affects the uptake and presumably translocation of several resources (Freschet *et al.* 2018). The more a clonal plant functions in an integrated manner, the more it should regulate its growth as a unitary plant. Accordingly, specialisation of integrated ramets to locally abundant nutrients by increased allocation to roots has been shown (Stuefer *et al.* 1998) and this response is analogous to root foraging in nutrient rich patches by parts of single root system (Giehl and von Wirén 2014). Therefore, clonal foraging for nutrients may be an additional or alternative (Weiser *et al.* 2016) way of root nutrient foraging (Zhang *et al.* 2022). However, morphological specialisation of ramets may not be beneficial if local conditions are likely to change or if the connection between ramets is likely to be severed.

Conceptually, in a clonal plant, information about local conditions can be integrated with information about other ramets and the overall status of a clonal system, and the local response can be adjusted accordingly (de Kroon *et al.* 2009). A nice example of such local and whole-plant signal integration in a unitary plant has been described for the regulation of nitrate uptake (Ohkubo et al. 2017; Oldroyd and Leyser 2020).

Plant competition and clonal integration

" *Resource competition is the process by which two or more individuals acquire resources from a potentially common, limiting supply.*" *(Craine and Dybzinski 2013)*

Heterogeneity in both light and belowground resources can be caused by the abiotic environment or generated by plant interactions themselves (Chazdon and Pearcy 1991; Skálová *et al.* 1999; Herben 2004). In habitats of generally low productivity, spatial and temporal heterogeneity may be determined mainly by abiotic factors. In contrast, when belowground resources are generally abundant, vegetation itself may form strong gradients, especially in light availability, and competition then becomes the dominant factor affecting plant growth. Competition for resources is then the major driver of plant community structure and affects the performance of individual plant species (Goldberg 1990). This determines a plant economics spectrum ranging from species adapted to low resource levels, focusing on resource conservation, to species of highly productive habitats, focusing on rapid resource acquisition and competition (Wright *et al.* 2004; Reich 2014).

Competition belowground and aboveground differs, although pre-emption of resource supplies plays an important role in both cases (Craine and Dybzinski 2013). The supply of soil nutrients is not directional, and nutrients may be locally depleted. A higher root length density in a soil patch implies a better ability to pre-empt nutrients. High uptake capacity and plasticity in response to nutrient heterogeneity is thus predicted to benefit plants in competition (Vázquez De Aldana and Berendse 1997; Hodge 2004; Maire *et al.* 2009). In contrast, light supply is directional: leaves positioned higher will only reduce light supply to other leaves, and smaller plants will thus be disproportionately disadvantaged in size-asymmetric competition for light (Weiner 1990; Craine and Dybzinski 2013).

Plants display different responses to light quantity or quality and other cues indicating aboveground competition. Plants facing a hopeless battle, such as those from forest understory, respond to shade by increasing shade tolerance (Valladares and Niinemets 2008; Novoplansky 2009). In contrast, plants with the potential to win the battle, such as those from open habitats, typically lift their leaves, increase vertical growth and reduce branching to reach light quickly and overshadow their neighbours (Franklin 2008). In addition, in clonal or procumbent species, these responses may be altered or complemented by horizontal shade avoidance by increased allocation to lateral spread (van Kleunen and Fischer 2001; Gruntman *et al.* 2017) or active foraging for light by directional lateral growth (Novoplansky *et al.* 1990; Macek and Lepš 2003; Gottlieb and Gruntman 2022). Such a horizontal shade avoidance strategy may occur particularly when the chance of reaching the top of the canopy is low (Gruntman *et al.* 2017).

The ability of clonal species to support ramets growing under low resource availability suggests that clonal integration might help plants to cope with competition (both aboveground and belowground). Several studies have addressed this hypothesis, particularly in the context of invasive species

spreading from uncompetitive to competitive conditions (e.g. Yu *et al.* 2009; You *et al.* 2014; Wang *et al.* 2016), and these have been reviewed and extended by P Wang *et al.* (2021). Growth from open patches to neighbourhood of other species was facilitated by clonal integration in some species (Roiloa *et al.* 2010; Xiao *et al.* 2011; You *et al.* 2014; Li *et al.* 2019; P Wang *et al.* 2021), while not in others (Pennings and Callaway 2000; Peltzer 2002; You *et al.* 2014). In *Fragaria chiloensis,* a positive effect of integration on clonal fragments growing into competition was demonstrated, but no effect of clonal integration was observed when whole clonal fragments were under dense competition of a taller grass (P Wang *et al.* 2021).

On a plant community level, differences in mechanisms of resource exploration and exploitation may affect plant coexistence (Loreau and Hector 2001; Tilman et al. 2014). A recent study on four clonal species (Wang *et al.* 2024) showed that integration promoted performance in three of them in experimental communities, especially in communities of low density and under high nutrient availability. Moreover, integration decreased biomass of the neighbour species, unless the community was dense and diverse. These findings are consistent with the results from North America grasslands, where nitrogen addition increased performance of tall clonal species (Gough *et al.* 2012), with negative impacts on species diversity (Eilts *et al.* 2011; Gross and Mittelbach 2017). Therefore, clonal growth represents another important but neglected factor that possibly affects plant interactions and performance of species in plant communities (Zobel et al. 2010; Gross and Mittelbach 2017; Mudrák et al. 2017).

There are several possible mechanisms of clonal integration role in competition. Integration of ramets may allow clonal plants to cope with resource heterogeneity by support of ramets (temporarily) limited by nutrients or light. In the case of light competition, such support may partially compensate for its asymmetry (de Kroon et al., 1992). Supported shaded ramets might grow higher and have better chance to overgrow their neighbours and reach the light on the vertical gradient (as also suggested by P Wang *et al.* 2021). This may be the case in tall clonals rising in the North America grasslands after nitrogen addition (Gross and Mittelbach 2017). Alternatively, supported shaded ramets may grow horizontally through the shaded area to reach a gap in vegetation (Semchenko *et al.* 2010). The preferred direction of light foraging (vertical or horizontal), might depend on relative height of the plant and its chance to reach the top of canopy (Gruntman *et al.* 2017). However, spatial heterogeneity in resource availability is likely required for clonal integration to be helpful in competition (P Wang *et al.* 2021). This may be reached by either different ramet positions in heterogeneous vegetation or different access to resources due to differences in size, which may be particularly pronounced in the case of light.

However, support of resource-limited ramets may not be the optimal resource-sharing strategy in all conditions, and other patterns of resource sharing may occur (Pitelka and Ashmun 1985; Evans 1988).

For example, if the chance of future improvements in resource availability is too low, the costs of supporting resource-limited ramets may be higher than its benefits for the clonal plant as a whole. Based on early labelling studies of clonal integration, Pitelka and Ashmun (1985) suggested three different strategies of clonal growth – a strategy that emphasizes lateral spread and resource exploration, a strategy that emphasizes ramet maintenance and a strategy of space monopolisation emphasising extensive integration of ramets. Regarding mainly carbon translocation, competitive and cooperative response to local resource limitation has been proposed in analogy with branches of non-clonal plants (Novoplansky 2003; Kawamura 2010). Which resource-sharing strategy is preferable may be determined by the nature of resource distribution (Pitelka and Ashmun 1985; Hutchings and Price 1993; Gardner and Mangel 1999; Mágori and Oborny 2003). As part of the plant economics, resource translocation between ramets may also be related to the typical habitat productivity and the nature of plant competition in the environment. In addition, directionality of growth remains in developed ramets, and these are typically not isolated from other, developing ramets. Directionality therefore should be included into thinking about resource sharing strategies among established ramets.

Besides resource translocation, other features of clonal growth, such as specific ramet positioning or resource storage, may be important in competition. The wide spacing of ramets enables quick colonization and exploitation of open patches and may thus bring competitive advantages in vegetation of lower density (Schmid and Harper 1985; Lenssen et al. 2005; Zobel et al. 2010), whereas the aggregated distribution of ramets has been shown to be advantageous in dense vegetation without open patches (Schmid and Harper 1985) and promotes the coexistence of species by reducing the level of interspecific competition (Bolker et al. 2003). Building of clonal organs may be a disadvantage for the clonal plants early in their development, but may pay off later (Martínková *et al.* 2020). Effects of clonal growth on competition thus may change in time.

In summary, clonal growth is an important factor in plant competition. However, there are multiple clonal growth forms with different sets of clonal traits and their role in plant coexistence is not well understood. Clonal integration is a key clonal trait and species differ in their responses to resource gradients by translocating resources between ramets. Although there are some general effects of clonal integration on plant growth and at least few rules seem to be universal, the theoretical predictions of translocation costs and benefits need further validation. The observed variability in resource translocation may be partially caused by specific mechanisms of translocation of different resources, or by different strategies in species experiencing different conditions and selective pressures, but a comprehensive understanding is lacking. I will address these areas in this thesis.

Aims

My aim here was to contribute to the understanding of the role of clonal integration in plant performance and how it relates to plant competition. I asked the following questions, in order of increasing generalisation:

- Do effects of resource sharing in clonal plants under external gradients of resource availability follow the theoretical predictions? (Paper I and II)
- How does resource translocation under light heterogeneity change during ramet ontogeny? (Paper II and III)
- Do the resource sharing patterns differ among species in a predictable manner? (Paper III and IV)
- How does the clonal growth form affect species performance in communities? (Paper V)

To answer the first three questions, I used an experimental approach and investigated resource sharing under heterogenous light (Paper II and III) and nutrients (Paper I and IV) by growth and labelling experiments. I have focused on carbon and nitrogen as the main macronutrients, although I am aware of importance of the other resources for plant growth and functioning. I combined the external heterogeneity in resource availability and different ontogenetic stages of ramets to obtain a complex picture of clonal integration under natural conditions.

I started with a growth experiment on a model clonal grass, *Agrostis stolonifera*, under different patterns of nutrient heterogeneity, testing theoretical predictions of the benefits of resource sharing in ramet pairs. I found that the benefits were unexpectedly higher at higher levels of nutrient availability (Paper I).

After this initial work, I focused on resource sharing in more detail, tracing the transport of labelled carbon and nitrogen between parent and offspring plant parts. Together with my colleague Jan Jansa, we developed a methodology to trace labelled carbon and nitrogen in both directions and across multiple ontogenetic stages, and demonstrated the transition of resource sharing patterns through the plant ontogeny of *A. stolonifera* under light heterogeneity (Paper II).

The pattern of translocation observed in the model clonal grass led me to ask whether translocation differs between species from habitats with different levels of competition. Therefore, using the same method, I compared the resource sharing strategies of two stoloniferous Rosaceae species under light heterogeneity and showed that they have different translocation patterns. In addition, I aimed to summarise and conceptualise the possible resource sharing strategies (Paper III).

Subsequently, I focused on the interspecific comparison of nitrogen translocation in six stoloniferous species under nutrient heterogeneity (Paper IV). I tested the hypothesis that differences in resource

sharing may be determined by the form and level of competition typically experienced by the species. The results did not confirm the hypothesis, but again showed the presence of different translocation patterns between species.

Finally, I addressed the fourth question by analysing performance of species with different clonal growth forms in plant communities using data from the long-term biodiversity experiment in Jena. The results suggested that species with different growth forms complement each other in their resource use strategies (Paper V).

In the following section, I will present the aims and results of each paper in more detail, discuss the results across the papers and, eventually, come to general conclusions.

Research summary

Paper I Evidence for unexpected higher benefits of clonal integration in nutrient-rich conditions (Duchoslavová and Weiser, 2017)

Paper II The direction of carbon and nitrogen fluxes between ramets in *Agrostis stolonifera* changes during ontogeny under simulated competition for light (Duchoslavová and Jansa, 2018)

Paper III Strategies of resource sharing in clonal plants: A conceptual model and an example of contrasting strategies in two closely related species (Duchoslavová and Jansa, manuscript submitted to Annals of Botany)

Paper IV Nitrogen sharing strategies in six clonal species (Duchoslavová, manuscript)

Paper V Effect of clonal growth form on the relative performance of species in experimental communities over time (Duchoslavová and Herben, 2020)

Benefits of clonal integration under nutrient heterogeneity did not reflect the predictions (Paper I)

Models of the costs and benefits of resource translocation in a pair of ramets (Fig. 2) predict that translocation should be most beneficial for the entire clonal fragment when surplus resources are translocated from a resource-rich donor ramet to a highly resource-limited recipient ramet. Therefore, net benefits should be highest when the contrast in resource availability is high, with donor ramets in high and recipient ramets in low resource levels. In addition, resource translocation is predicted to be beneficial even under homogeneous environmental conditions when ramets differ in their uptake capacity, as the uptake capacity of ramets shifts their position along the resource availability gradient.

I tested these predictions in a growth experiment using pairs of mother and daughter ramets of *Agrostis stolonifera*. I used three levels of mother nutrient availability crossed with two levels of daughter nutrient availability, resulting in different levels of contrast between ramets and different total nutrient availability. I compared the growth of integrated ramet pairs with that of disconnected ramets under the same conditions to estimate the effects of resource translocation.

According to the expectations, the benefits of resource sharing for daughters were present in all treatments, including the homogeneous conditions. However, nutrient levels did not affect the benefits of resource sharing as expected, although the relationship between final biomass and resource availability in the model system was nicely consistent with the assumed saturation curve. Instead, the increase in biomass of daughters due to integration was greater at higher levels of their nutrient availability. Integration had no significant effect on the mothers, which were three weeks older and on average almost four times larger than the daughters at harvest. However, the effect of integration was

positive for the daughters and neutral for the mothers even when mothers had lower nutrient availability than daughters and were of comparable size.

I see three possible explanations for the unexpected results. First, a higher effect of integration in daughters at a higher nutrient level could be caused by the multiplicative nature of growth. If the conceptual relationship (Fig. 2) represents increase in growth rate rather than in absolute biomass, then exponential relationship of ramet size in time could cause the observed pattern. Even if the benefits of resource translocation for the growth rate are the same for ramets with different initial resource levels, they are translated into different benefits for the final size of the ramets (Fig. 4). This effect on size is opposite to the effect of decreasing benefits for the growth rate with increasing resource levels (Fig. 2). Consistently with this explanation, using a logarithmic transformation mitigates the effect of the daughter's nutrient level. This explanation suggests that the conceptual cost-benefit model should be based on growth rate rather than absolute biomass increase, and that caution should be taken when summing costs and benefits for ramets of different resource levels and sizes to produce a net benefit for a clonal fragment (Eriksson and Jerling 1990; Caraco and Kelly 1991).

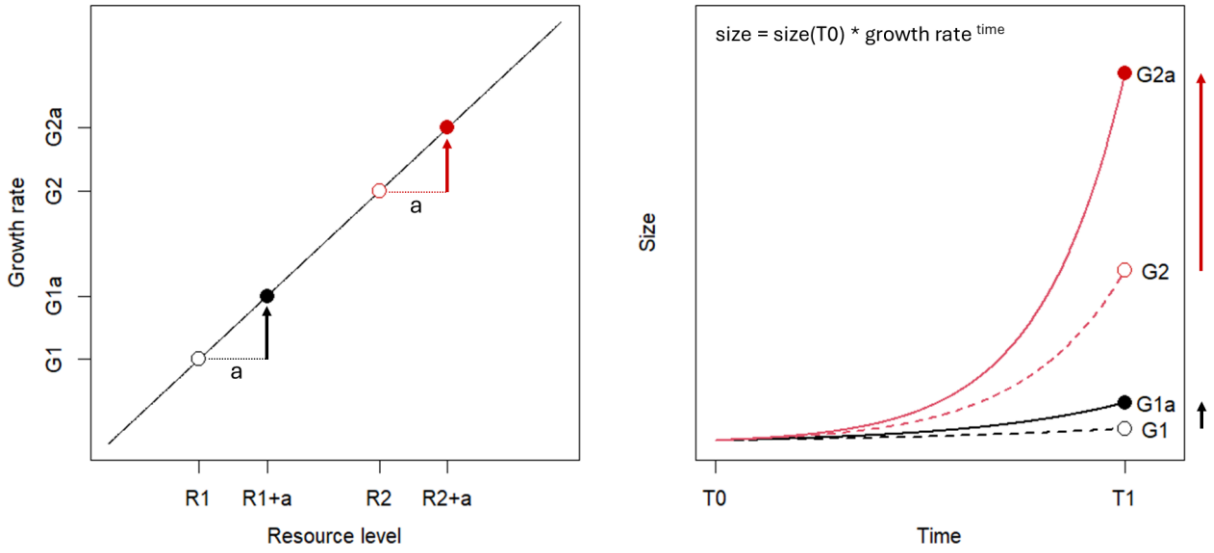

Figure 4 *Hypothetical benefits of resource translocation for growth rate (left) and ramet size (right). If the growth rate increases linearly with resource level, translocation of a given resource amount (a) to ramets with different initial resource levels will result in the same benefits for growth rate (indicated by arrows). However, the benefits for the ramet size will be higher in ramets with higher initial resource level, as indicated by arrows.*

Second, nutrient translocation may be higher to daughters growing in relatively higher nutrient levels. Consistent with this explanation, the relative allocation of biomass to daughters due to integration was highest when mothers grew in pure sand. Moreover, the above-mentioned logarithmic transformation accentuated the effect of the mother's nutrient level on benefits of integration, with mothers in nutrient-poor conditions supporting their daughters the most. Similar patterns of nutrient translocation have been described previously (Alpert 1996; Sun *et al.* 2011), and were hypothesised to be caused by

higher transpiration of relatively larger daughters and translocation of nutrients along the transpiration stream.

Third, I speculated that translocation of photosynthates rather than nutrients would reveal the observed pattern, i.e. enhanced growth of daughters not limited by their local nutrient conditions. Translocation of photosynthates could also explain the unexpectedly higher allocation to roots in integrated daughters. Alternatively, the unexpected response in root allocation could be an indication of changing direction of nutrient translocation after daughter establishment, with daughters translocating nutrients back to mothers.

All these considerations led me to further focus on tracing resources that are actually translocated (Papers II, III and IV), changes in translocation during ontogeny (Papers II and III) and comparing nutrient translocation across species (Paper IV).

Carbon and nitrogen translocation changes during ontogeny and its response to light heterogeneity differs between species

Resource translocation is particularly important for new, developing ramets. However, exchange of resources may be maintained among developed ramets, and it can be induced or enhanced by heterogeneity in resource availability. Such heterogeneity can be generated by plant competition, creating strong horizontal gradients in light availability for young or short plants. Clonal integration may help plants to cope with such heterogeneity and partly mitigate the asymmetric nature of light competition.

To disentangle the role of clonal integration in light competition, my question here was how resource translocation between mother and daughter ramets changes under light heterogeneity and during ramet ontogeny. I expected that the initial high translocation to daughters would generally decrease with time, but that some level of carbon translocation to shaded ramets would persist after daughter ramet establishment. To test this, I examined patterns of resource translocation first on *Agrostis stolonifera* and then on two closely related Rosaceae species, *Fragaria viridis* and *Potentilla reptans*.

The nitrogen and carbon economy of plants is tightly linked. For example, energy gained from carbon is needed for nitrogen uptake, and nitrogen is an important component of chlorophyll. For this reason, I decided to measure translocation of both elements in these experiments. I assessed resource translocation patterns between ramets at a given developmental stage directly by tracing stable isotopes of carbon and nitrogen in both directions. I examined three developmental stages for *Agrostis* and two for the Rosaceae species.

Agrostis stolonifera (Paper II)

At the very beginning of daughter ramet rooting, *Agrostis* daughters were highly dependent on nitrogen imported from mothers, as expected, but at the same time they were independent in carbon acquisition and even exported carbon to mothers. After two weeks, at the time of daughter vigorous growth, they became independent in nitrogen uptake, but carbon translocation was directed to daughters. Therefore, their roots and emerging tillers probably formed a strong sink for carbon. At the time of final harvest, net nitrogen translocation was directed slightly to mothers, and it appeared to be more so when the mothers were not shaded. Surprisingly, carbon translocation was directed from shaded mothers to established daughters. Therefore, it appeared that mothers in the light harvested nitrogen via established daughters, whereas mothers in the shade reallocated carbon to daughters. Absolute amounts of translocated carbon and nitrogen did not decrease with time, contrary to the expectations. They, however, accounted for smaller proportions of the total assimilated resources at later stages of development. Integration had a positive effect on daughter growth with no significant effect of shading, probably reflecting the translocation pattern at the beginning of daughter ramet development.

The results contrasted with the predicted higher translocation of carbon to shaded ramets. I speculate that shading selectively inhibited the growth of new tillers in the older part of the clonal fragments, thus weakening the main sink for carbon and nitrogen translocated from the daughters. If so, the reduced external availability of light to mother ramets led to their reduced demand for resources, contrary to common expectations.

Fragaria viridis and *Potentilla reptans* (Paper III)

The observed translocation pattern in *Agrostis* led me to the idea that a translocation strategy might be related to the level of competition for light typically experienced. I expected that plants experiencing asymmetric competition from typically taller surrounding vegetation would not maintain older shaded ramets by translocating resources, but rather invest in the growth of younger ramets and their clonal growth, i.e. show a translocation pattern consistent with the horizontal shade avoidance strategy.

To build on this idea, I applied the same experimental approach to compare resource translocation in two Rosaceae species from different habitats with contrasting productivity. For logistical reasons, I reduced number of shading treatments to three (skipping the treatment with both ramets shaded) and number of ontogenetic stages to two (two and eight weeks after daughter rooting initiation).

Young daughters of both species were supported both by carbon and nitrogen at the early developmental stage. However, carbon translocation to mothers increased when they were shaded. At the later developmental stage, the two species responded differently to shading. The species of low-productivity habitats, *Fragaria viridis*, translocated more carbon to shaded ramets (both mother and

daughter). In contrast, the species of high-productivity habitats, *Potentilla reptans*, did not support shaded mother ramets by carbon at all. Nitrogen translocation remained mainly acropetal in both species.

The results demonstrated different translocation strategies in two closely related species of similar growth habit. These strategies may be linked to the habitat conditions experienced by each species, but this hypothesis needs to be further tested by comparing more species. I also speculate that *Potentilla* would respond in a different way to short term shading, as it tended to support the mothers that were shaded for two weeks, but not those that were shaded for eight weeks. Switching in response to shade when it lasts too long was also reported for the (vertical) shade avoidance strategy (Franklin 2008).

The three species together

In summary, only one species, *Fragaria viridis*, showed the commonly expected carbon translocation pattern equalising the environmental gradient in light availability. The other two species, *Potentilla reptans* and *Agrostis stolonifera*, showed a different response to shading, with no carbon translocated from established daughters to shaded mother ramets. Moreover, they tended to translocate carbon from the shaded mother ramets to the daughter ramets, more so in *Agrostis* than in *Potentilla*. I suggest that this translocation pattern is consistent with the horizontal shade avoiding strategy.

In addition, the results demonstrate the necessity of bidirectional tracing of resource translocation to estimate net flows or resources between ramets. Even high translocation in one direction can be accompanied by an equally high reverse translocation. However, only few studies have examined translocation between ramets in both directions (Alpert 1991, 1996; Pinno and Wilson 2014; Dong *et al.* 2022).

Conceptual model of resource sharing (Paper III)

The results of my translocation experiments clearly indicated that the pattern of resource translocation in clonal plants is not a simple function of resource availability and that we need to take different translocation strategies into account. I aimed to summarise possible translocation strategies in a conceptual analysis presented in the Paper III (Fig. 5). I suggested that the commonly considered 'equalisation' strategy is only one of several possible strategies. Under certain conditions, a strategy emphasising acropetal movement and exploration of new areas (an 'acropetal translocation strategy') or a strategy of accumulating resources in older ramets (an 'extended hand strategy') may be preferred. The optimal strategy may be determined by environmental conditions, such as resource availability and level of light competition. The equalisation strategy may be preferred when all individuals have a chance to reach the top of the canopy since it enables the maintenance of established ramets. The acropetal translocation strategy may be preferred if there is major horizontal heterogeneity in light availability, as it might enable rapid spread to new, potentially unshaded patches.

The extended hand strategy may be preferable when concentrating resources in the mother ramet brings benefits for the entire clonal fragment. I expect this strategy to be particularly effective for exploration of soil-borne resources which might get depleted by older ramets.

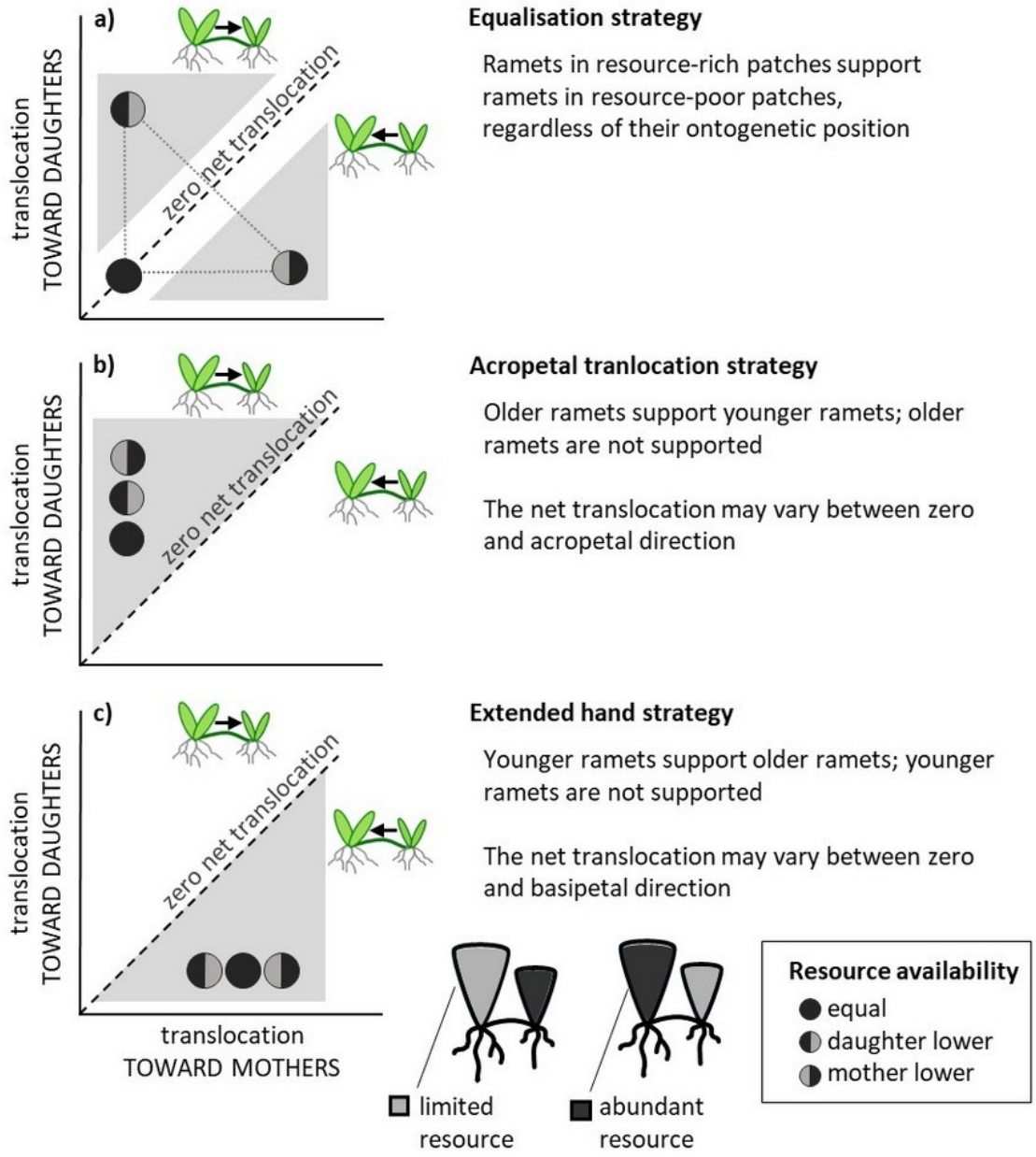

Figure 5 *Hypothetical* ***two-way translocation plots*** *for the proposed resource-sharing strategies between mother and daughter ramets in the later developmental stage. The dashed line indicates zero net translocation. Translocation toward daughters prevails in the zone above the dashed line, while translocation toward mothers prevails in the zone below the dashed line. Grey triangles indicate zones in which the values may range.*

With a focus on nitrogen

Although I focused mainly on the carbon translocation in response to shading in the two labelling experiments, I also labelled the nitrogen to get a more complex picture of plant resource economy through ramet ontogeny. *Agrostis* daughter ramets appeared to be independent in nitrogen uptake two weeks after beginning of rooting under homogeneous conditions, and later they even translocated nitrogen back to the mothers. In contrast, daughters of *Potentilla* and *Fragaria* were still supported by nitrogen eight weeks after beginning of rooting. Therefore, they have not yet reached the stage of nitrogen independence, either because they were harvested too early or because this stage was inhibited by clonal integration (Roiloa 2019; Xi *et al.* 2019). The continuous support of daughters by nitrogen may reflect translocation of nitrogen from mothers to new unrooted stolons in these species (Alpert 1999). However, I did not separate the rooted rosettes from the non-rooted stolons, and so I cannot test this prediction.

The nitrogen translocation to barely rooted daughter ramets should be mainly controlled by the strength of the sink formed by the ramets. Accordingly, the relative size of the daughters was the main determinant of nitrogen translocation to the daughters with undeveloped roots in all three species, with the proportion of nitrogen exported corresponding well to the relative daughter size (Fig. 6). This straightforward relationship disappeared at later developmental stages. Therefore, the higher acquisition of water and nitrogen by more developed daughters made the source-sink relationship more complex.

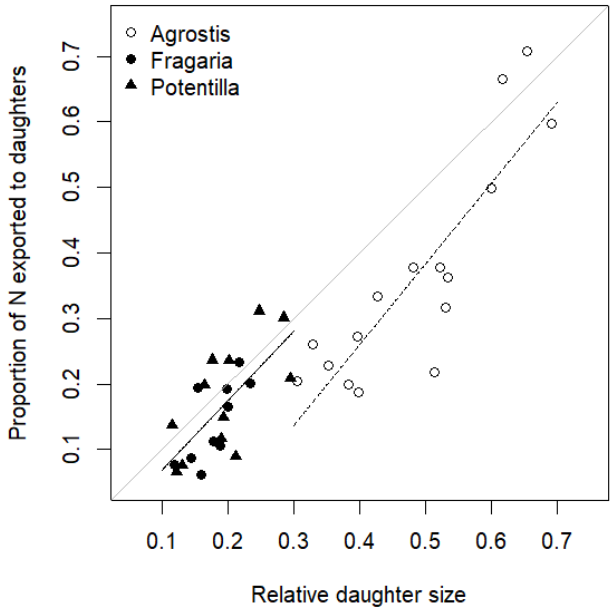

Figure 6 *Relationship between relative daughter size and proportion of labelled nitrogen exported from mothers to daughters in the three species. Labelling was performed at the time of rooting for* Agrostis *and two weeks after rooting initiation for* Fragaria *and* Potentilla*, at which time all the species had undeveloped roots.*

The results of the *Agrostis* translocation study also allows me to relate them to the unexpected results of the growth experiment in Paper I. I suggested that the higher growth benefits of integration for daughters at high nutrient level might have been caused by the multiplicative nature of growth, by translocation of photosynthates, or by higher translocation of nutrients to daughters at high nutrient level. The labelling approach confirmed the hypothesised carbon translocation to daughters under homogeneous conditions as well as higher nitrogen translocation to relatively larger daughters. However, I can't deduce what the translocation would be under nutrient heterogeneity. Moreover, I observed the suggested daughter specialisation for nutrient acquisition in the later developmental stage.

Species differ in nitrogen translocation, irrespective of nutrient heterogeneity (Paper IV)

Nitrogen is often a limiting factor for plant growth, and its availability is a major determinant of level of competition. It is also an important component of a plant economics spectrum ranging from species adapted to low resource levels, focusing on resource conservation, to species of highly productive habitats, focusing on rapid resource acquisition and competition (Reich 2014). In clonal plants, patterns of nitrogen translocation between ramets may be part of plant nitrogen economics, and, as such, may also be related to the typical availability of nitrogen. In nutrient-poor habitats, extensive nutrient sharing balancing resource availability may be particularly important to maintain established ramets and to capture soil resources from a larger area. On the other hand, nutrient sharing between established ramets may not be beneficial in productive habitats where mineral nutrients are not limiting and competition for light is the main determinant of plant growth.

I tested the proposed nutrient sharing strategies on nitrogen translocation in six stoloniferous species that occur in habitats of varying productivity. They were either grown in a homogeneous nutrient-poor treatment or the mother part in a nutrient-poor and the daughter part in a nutrient-rich treatment. I traced the translocation of nitrogen in both directions using stable isotope labelling, one month after the start of the rooting of the daughter ramets.

Surprisingly, I found no effect of nutrient treatment on nitrogen translocation. Instead, each species translocated nitrogen either acropetally, basipetally, or equally in both directions. There was no relationship between the direction of translocation and the productivity of the species' habitats. However, net translocation seemed to be related to the relative size of daughters across species, and also intraspecifically in *Veronica officinalis*.

In line with my previous findings, the results of this experiment suggested that the relative size of plant parts is an important determinant of the strength of the sink for nitrogen they form, and that the growth habit of a species can affect its nitrogen translocation. Under certain conditions, such internally induced source-sink relationships may dominate over external nitrogen heterogeneity. *Fragaria viridis*

and *Potentilla reptans* were used in both types of labelling experiments (i.e., Papers III and IV) and their nitrogen translocation pattern was consistently acropetal. *Agrostis stolonifera* was not used in the nitrogen translocation experiment, but it could probably be added to the list of species with a basipetal nitrogen translocation pattern. These results confirmed the extended hand strategy proposed in my conceptual model (Paper III) for some species. Despite the basipetal nitrogen translocation, daughter ramets of these species may still benefit from integration due to early translocation or translocation of photosynthates.

Effect of clonal growth form on the relative performance of species in experimental communities over time (Paper V)

Although all plants use similar types of resources, plant species differ in their mechanisms of resource exploration and exploitation. Therefore, available resources may be used more completely by mixtures of plants with different resource-use niches. At the same time, the performance of individual species in communities is modified by traits affecting efficiency in the uptake and use of limited resources. Here, I hypothesised that clonal growth represents an important trait that affects the performance of species in communities. Specifically, I expected clonal species to i) perform worse in the early stages of community development but better in later stages, ii) perform better in communities with low proportions of clonals, and iii) I expected long-spreading clonals to perform better in communities with lower densities and short-spreading clonals to perform better in communities with higher densities. To test these hypotheses, I analysed the effect of different clonal growth forms on the relative performance of plant species in communities of the Jena Biodiversity Experiment over a ten-year period.

The clonal growth form did not affect the relative performance in the early stage of communities and none of the growth forms gained clear dominance during the experimental period, which did not support the first hypothesis. The stoloniferous species performed better in communities with a higher proportion of nonclonals, partly supporting the second hypothesis and suggesting complementarity in the light exploitation strategies of nonclonal and stoloniferous species, which are generally of low stature and may forage for light by lateral growth. The species with long rhizomes generally performed slightly better than the others, particularly in communities of low diversity (and density). However, there was no effect of community diversity on performance of short rhizome clonals. This partly supported the third hypothesis and suggested that relatively fast-spreading long rhizome species can colonise space effectively when shoot density is low. In addition, recent work has shown that the benefits of clonal integration are higher at lower vegetation diversity and density, which may generate more heterogeneity (J Wang *et al.* 2021; Wang *et al.* 2024). Therefore, resource sharing may have been beneficial for the long rhizome species under higher heterogeneity, either due to their better ability to

support shaded ramets, or due to their better ability to explore and exploit vegetation gaps by the acropetal translocation strategy.

In summary, the results of my study suggested that species with different growth forms complement each other in their resource use strategies. They also showed the necessity of distinguishing among different clonal growth forms when analysing effect of clonality on plant performance.

General conclusions

Clonal integration has fascinated plant ecologists for decades. My main contribution here is to the mechanistic understanding of carbon and nitrogen translocation between ramets. I combined effects of external resource heterogeneity and differences in ramet size and found distinct patterns of carbon and nitrogen translocation across different stoloniferous species. I also attempted to understand more about the 'source-sink relationships' so often used in the context of clonal integration. My results confirmed the expected universal support of developing young ramets, which clearly form strong sinks for both nitrogen and carbon. However, I suggest that mechanisms of nitrogen and carbon translocation differ in later developmental stages of ramets. The strength of the sink for nitrogen was proportional to ramet size in young ramets with undeveloped roots. Moreover, ramet relative size seemed to be a promising predictor of later nitrogen translocation pattern across species, which was surprisingly not affected by nutrient heterogeneity. In contrast, carbon translocation was driven by external availability in light, although different species differed in their willingness to send carbon to older ramets. I speculate that species-specific regulation of growth response under different light conditions affects strength of carbon sinks and determine differential translocation patterns.

I want to point out that integration of a plant body is quite well-studied in nonclonal plants. Clonal integration is different due to the presence of multiple rooting points, which change the direction of xylem flow in particular, and also the allocation of plant biomass. This is likely to change the transport of water and nutrients, but perhaps not so much for carbon. Therefore, more comparisons with carbon transport in nonclonal plants may bring valuable insights into the clonal integration research.

Mechanistic insights are hard to obtain from commonly performed growth experiments, which do not separate early and late effects of clonal integration. Results from labelling and growth approaches often do not match, and I suggest that this discrepancy is caused by dominant effect of the early translocation on the final performance of ramets in usually rather short-term experiments. However, labelling studies comparing both directions of possible translocation are so far rare, and studies tracing multiple resources are even rarer. I particularly encourage further experiments using a comparative approach, as little is known about the drivers of interspecific variation in clonal integration and the links between plant economics strategies and translocation patterns. The relationship between habitat productivity, which underlies the plant economic spectrum, and translocation patterns requires further attention. Understanding this could help to better explain the role of clonal species in plant communities.

The understanding of the role of clonal integration in the context of plant communities and real-life interactions is also very important. I contributed to this field by the analysis of performance of clonal growth forms in the Jena biodiversity experiment, which suggested complementarity of different growth forms in their resource use strategies. In this context, it is important to consider that resource

translocation through clonal integration is not the only specific feature of clonal species. For example, clonal organs can be used to store carbohydrates, which may be expensive to build at first, but useful later (Martínková *et al.* 2020). In the short term, integration may have clear benefits for clonal plants, especially if the donor species is growing under high resource availability. However, in the long term, the storage of resources and the expansion of the acquisition area may become more important. Therefore, although both approaches are important, deriving competitive effects of clonal growth from mechanistic understanding of clonal integration is challenging.

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Paper I

Evidence for unexpected higher benefits of clonal integration in nutrient-rich conditions

Jana Duchoslavová[1], Martin Weiser[1]

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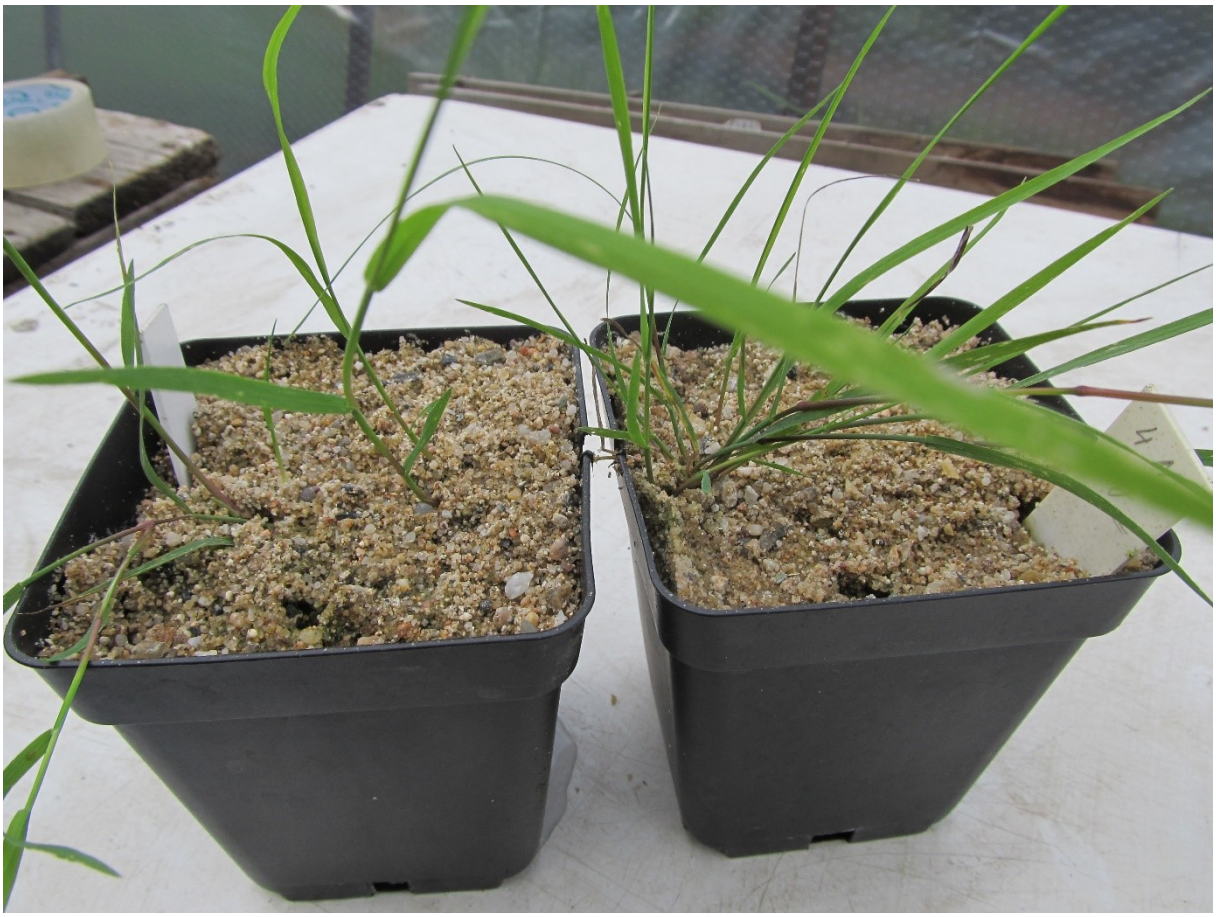
[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 43 Praha 2, Czech Republic



Abstract

Physiologically integrated clonal plants cope better with spatial heterogeneity due to their ability to share resources among ramets. According to theoretical predictions and experimental evidence, such benefits of resource sharing should increase with higher patch quality of an exporting ramet and lower patch quality of an importing ramet. This study investigated the effect of spatial heterogeneity in nutrient availability on benefits of clonal integration under plausible scenarios of clonal spread, in which more developed ramets give rise to new ones. Pairs of mother and daughter ramets of a stoloniferous grass, *Agrostis stolonifera*, were grown in various nutrient conditions. Disconnected pairs of ramets were used as controls. Results showed considerable benefits of integration for developmentally younger daughters and no costs for older mothers in all treatments. Surprisingly, benefits of integration were more pronounced in nutrient-rich daughters, and allocation to integrated daughters decreased with increasing nutrient level of mothers. In addition, integration in general increased root-to-shoot ratio of daughters. One possible explanation of the observed patterns may be prevailing translocation of photosynthates rather than nutrients. Daughters also responded to nutrients by changes in clonal architecture. Number of stolons increased, and maximum stolon length decreased in high nutrient levels. Integration increased maximum stolon length in small daughters. The architectural responses are generally in accord with the foraging behaviour concept. Overall, our results suggest that resource translocation within a clonal fragment need not be easily predictable from a gradient of resource availability.

Keywords

clonal integration, spatial heterogeneity, patch contrast, resource level, foraging, *Agrostis stolonifera*

Introduction

Physiological integration of ramets enables clonal plants to share resources from different sites. Translocation of water, nutrients and photosynthates between interconnected ramets of clonal plants has been demonstrated in numerous experimental studies (Noble and Marshall 1983; Chapman et al. 1992; de Kroon et al. 1996). Benefits of resource sharing have been reported mainly in heterogeneous conditions, in which neighbouring ramets face contrasting resource availability (Alpert and Mooney 1986; Alpert 1991). In addition to resource sharing, specialization of ramets in uptake of locally abundant resources can amplify the positive effect of integration on performance of a whole clonal fragment in heterogeneous habitats (Alpert and Stuefer 1997). Selective placement of ramets into favourable sites can help plants explore spatial heterogeneity effectively (Sutherland and Stillman 1988).

Increasing benefits of resource sharing in heterogeneous habitats can be explained by a simple theoretical framework (Caraco and Kelly 1991; Dong et al. 2015). Benefits and costs of resource sharing in a pair of ramets are affected by a resource availability-ramet performance relationship as well as by a position of both ramets on the resource availability gradient (Fig. 1). Performance of individual ramets in response to resource availability can be expected to rise steeply in low resource level and level off at maximum in high resource level when another resource becomes limiting. Considering such a resource availability-performance relationship, both benefits of import of a given amount of resource and costs of the resource export for a ramet decrease with higher levels of resource availability. Thus, net benefits of clonal integration can be expected to increase with higher resource availability of an exporting and lower resource availability of an importing ramet. In other words, the resource-rich donor ramet becomes saturated by the resource and therefore pays little to export the surplus resource, whereas any resource support is translated into enhanced growth in the resource-limited recipient ramet.

An indication of increasing effect of physiological integration with increasing contrast in resource availability can be found in experimental studies. For example, benefits of clonal integration for water-limited ramets increased with the severity of this limitation in *Fragaria orientalis* (Zhang et al. 2008). In experiments with ramet pairs grown in reciprocal gradient of light and nitrogen availability, the effect of integration on biomass was revealed only in highly contrasting conditions in *Fragaria chiloensis* (Friedman and Alpert 1991) and *Potentilla anserina* (Wang et al. 2011). In addition to a net effect of clonal integration, ramet specialization for uptake of locally abundant resource may increase with increasing contrast in resource availability (Roiloa et al. 2007; Guo et al. 2011; Wang et al. 2011).

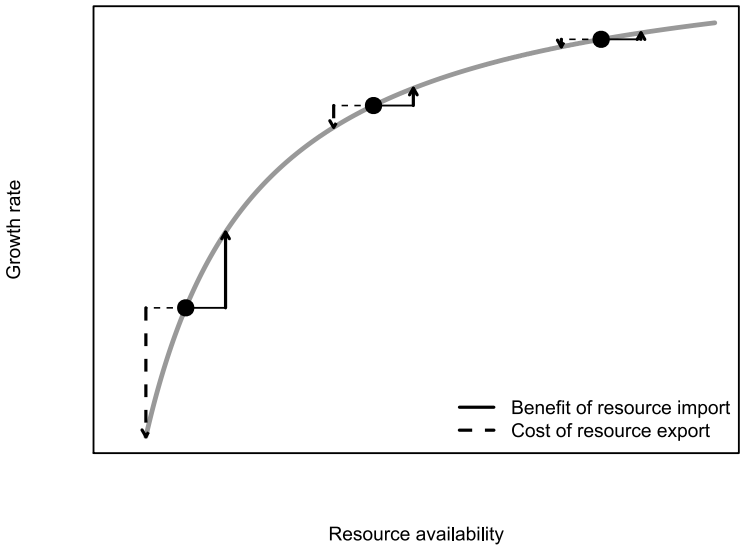


Figure 1 *Benefits and costs of resource sharing according to the theoretical predictions. Both export and import of a given amount of resource affect a ramet less the higher the resource availability is.*

Regardless of habitat heterogeneity, resource translocation is of particular importance for developing young ramets supported by established parental ramets (Marshall 1990), because the different ability of the ramets to take up resources alters their internal resource level (Dong et al. 2015). In terms of the theoretical framework, the low uptake ability shifts ramet position on the resource availability gradient to lower values. Therefore, benefits of integration can also be expected in homogeneous conditions for ramets differing in their uptake ability. Resource translocation among ramets of unequal size and developmental stage is likely to be very common in nature whenever a clonal fragment grows. However, experimental studies on clonal integration have so far used mostly pairs of ramets of similar size (see Dong et al. 2015; Stuefer et al. 1994 for some exceptions).

In addition to benefits of resource sharing, clonal growth potentially enables plants to explore heterogeneous habitats by adjusting clonal architecture in response to experienced conditions. According to the concept of foraging in clonal plants (Sutherland and Stillman 1988; de Kroon and Schieving 1990), plants growing in resource-poor patches should invest in escaping from the patch by producing long spacers, whereas plants positioned in resource-rich patches should invest in exploiting local resources by positioning daughter ramets close to the original patch. Although the effectiveness of such resource exploration in real habitats was questioned (Cain 1994; Oborny 1994), experimental evidence of such foraging behaviour exists (Slade and Hutchings 1987; Macek and Lepš 2003). Furthermore, information about spatial heterogeneity may be integrated in interconnected clonal fragments, and the foraging response of a ramet can therefore be modulated by integration with other ramets (Louâpre et al. 2012).

We investigated effect of ramets' nutrient level on benefits and costs of clonal integration in pairs of ramets of different developmental stage and body size. We use pairs of mother and daughter ramets of

a clonal grass *Agrostis stolonifera*. Because daughter ramets were considerably smaller than mother ramets at the beginning of the experiment and their roots were less developed, we assumed that photosynthates and nutrients were translocated predominantly in the direction towards daughters even if the daughter ramets were in the same or higher nutrient level than the mother ramets. In addition, we explored if the foraging behaviour in response to nutrient availability occurs in *Agrostis stolonifera*. To estimate plant architectural responses, we took a maximum stolon length as a measure of investment to habitat exploration and number of stolons rooting in a pot and forming a clump as a measure of exploitation of local resources.

Stemming from the theoretical framework, we tested these specific predictions about effects of integration on plant biomass: (i) benefits of clonal integration and its relative importance for daughters will decrease with their increasing nutrient level; (ii) benefits for daughters will increase and/or costs of clonal integration for mothers will decrease with increasing nutrient level of mothers; and (iii) in homogeneous conditions, effect of integration on daughters will be positive due to differing developmental stages of mothers and daughters. As to specialisation of ramets to uptake of a particular type of resource, we predicted that (iv) clonal integration will stimulate allocation to roots in mothers to cover nutrient demands of daughters, especially when daughters experience low nutrient level, and (v) clonally integrated daughters will allocate less biomass into roots because their nutrient demand will be partly covered by translocation from mothers. This effect will be most pronounced in nutrient-poor daughters integrated with nutrient-rich mothers. With respect to the clonal architecture of daughters, we predict that (vi) maximum stolon length will be higher in nutrient-poor conditions, (vii) the number of stolons will be higher in nutrient-rich conditions, and (viii) the architectural responses may be modulated by integration with mothers.

Methods

Species

Agrostis stolonifera is a clonal grass forming long stolons, common in mesic and wet grasslands and river banks (Kik et al. 1990; Kubát et al. 2002). It usually reproduces clonally; some clones (genotypes) occasionally propagate through seeds. Newly established ramets produce vertical tillers. When these tillers reach a certain critical length, they bend groundwards and become stolons that shed their leaves and root at some of their nodes. Throughout the Czech Republic, *Agrostis* genotypes substantially vary in their ploidy level.

Plant material for the experiment originated from a single hexaploid clone of *Agrostis stolonifera* collected in a field and grown in a common garden since 2010. None of the plants flowered in the experiment. Because we were unable to predict the fate of each tiller, we refer to all stems as "stolons".

Initial cultivation

On 5 June 2013, stolons of *A. stolonifera* were cut from the plants in the common garden and put on trays with wet sand. On 3 July, the nodes with developed roots and leaves were isolated and planted in 1-liter pots filled with washed sand and supplied with slow release fertilizer (Substral Osmocote Grass, percentual nutrient content: N, P, K, Mg, S-23, 5, 10, 2, 9) according to their experimental nutrient level (Fig. 2). Pots were situated in a plastic film greenhouse and watered twice a day with tap water. The initial impurity of sand and tap water was later estimated to equal 0.096 g (s.e.: 0.024 g) of fertilizer.

After three weeks of cultivation of these mother plants, the longest stolon of each plant was placed in an adjacent pot with sand to form a daughter plant. The initial size of a mother plant was measured as the length of all stolons forming the plant without the longest one, and the initial size of a daughter plant was estimated as the length of the longest stolon. The daughter plants were supplied with fertilizer according to their experimental nutrient level immediately after planting.

Experimental design and measurement

We used factorial design with three nutrient levels for the mother plants (0, 1.5 and 3 g of the fertilizer), two nutrient levels for the daughter plants (0 and 1.5 g of the fertilizer) and two levels of stolon connection—i.e., stolon between mother and daughter ramet left intact or severed (the control treatment). There were 14 replicates for each combination of factors.

In addition, we estimated growth response of *A. stolonifera* to a gradient of fertilizer dosing. For this purpose, we used the control mother plants together with additional plants grown in the same way as the control mother plants. To evenly cover the gradient of fertilizer, the additional plants were given 0.75, 2.25, 3.75, 4.5 and 5.25 g of the fertilizer with 10 replicates for each fertilizer dosage.

Stolons connecting mother and daughter plants were cut in the control treatment after one week of rooting (on 1 August). After one month (on 2-4 September), all plants were harvested. By that time, daughters had on average 11.8 stolons (i.e., stems) of a mean length 24.7 cm. Stolons of daughter plants were counted, and their lengths were measured as the distance from the rooting point to the tip of the most distant leaf. Biomass of all plants was then separated into shoots and roots, dried to constant weight (65° C, 3-4 days) and weighed.

The collected data are available as supplementary material (Online Resource 1).

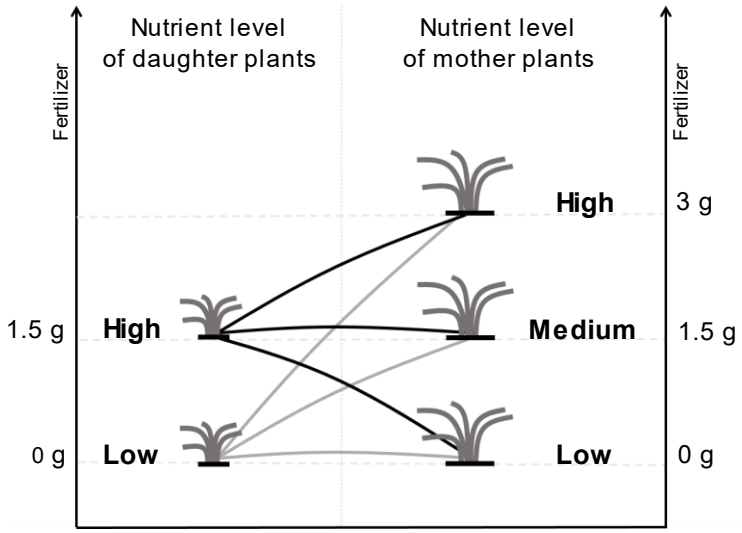


Figure 2 *Experimental design: Integrated pairs of older mother and younger daughter plants with two nutrient levels for daughters and three nutrient levels for mothers were used. Control treatment consisted of pairs of plants with severed connection grown in corresponding nutrient levels.*

Statistical analyses

Four daughter plants died during the experiment. All these daughter plants had severed connections and were fertilized by 1.5 g of fertilizer. We excluded these plants and their mothers from the analyses.

All statistical analyses were performed in the R statistical environment (R Core Team 2015; R version 3.2.3).

The growth response of *A. stolonifera* to different fertilizer dosages was modelled using Michaelis-Menten equation because it describes saturation dynamics. We used the *mle2* routine from the *bbmle* package (Ben Bolker and R Development Core Team 2016, ver. 1.0.18) to fit the equation parameters according to maximal likelihood. Besides (i) the plant size asymptote and (ii) fertilizer amount needed to reach half that size, the model fitting procedure included estimation of (iii) the amount of nutrient residues in the washed sand and in the tap water we used for watering and (iv) biomass variance linearly increasing with the biomass mean.

Similarly, we modelled root/shoot ratios of the plants as a response to fertilizer dosage. In this case, we used generalized additive modelling (Gaussian family, dimension of basis=3). The *gam* routine form *mgcv* package (Wood 2011) was used for additive modelling.

The effect of connection on plant biomass and root/shoot ratio was tested by a linear model with connection treatment, nutrient level of mothers and nutrient level of daughters as the main factors and the initial size of plants as a covariate. Separate analyses were performed for mother and daughter plants. Root/shoot ratios of all plants and biomass of the mother plants were log-transformed prior to the analyses, whereas biomass of the daughter plants was square root transformed to meet assumptions

of the models. Initial size of plants was transformed in the same way as the response variable in each model.

Variation explained by the connection treatment was determined in a set of separate linear models for each combination of daughter and mother plant nutrient level. Biomass (square root transformed) in response to initial size of plants (square root transformed) and connection treatment was modelled for each combination. We took only the information about explained variations from these models.

Relative biomass allocation of whole pairs of plants into daughter plants was analysed by a linear model with a daughter-to-mother biomass ratio as a response variable, nutrient level of daughters, nutrient level of mothers and connection treatment as main factors and initial size of both mother and daughter plants as covariates. Response variable and covariates were log-transformed.

Architectural responses of daughter plants were estimated using maximum stolon length and the number of stolons as response variables. Maximum stolon length was used as a proxy of the exploration activity of plants, whereas the number of stolons describes a clump density, which may reflect the level of exploitation of local resources. However, plastic responses in allocation have to be distinguished from pure allometric effects since producing absolutely more shoots in high nutrient level can be explained simply by faster growth in better conditions (Huber and Stuefer 1997, Weiner 2004). We avoid this effect by relating the architectural responses to plant size (aboveground biomass) and restricting the comparison to range of sizes represented by plants from both nutrient levels (Weiner 2004). Thus, a three-way ANCOVA was used with aboveground biomass of daughters, nutrient level of daughters and connection treatment as explanatory variables. The range of aboveground biomass was restricted to cover only values with plants from both nutrient levels represented (excluding 29 smallest or largest plants). Response variables as well as aboveground biomass were log-transformed. Seven plants were excluded from the analyses of architectural responses because they lost and subsequently regenerated whole shoots during the experiment, which resulted in deviations in their architectural traits.

Results

Growth response to fertilizer

Plants involved in the growth response experiment responded to higher fertilizer amounts by bigger biomass accumulation and lower biomass allocation to roots (Fig. 3). This pattern held up to approximately 3 g of fertilizer, reaching almost 6 g of biomass and 0.2 in root/shoot ratio. There was not any apparent further increase in plant biomass or decrease in root/shoot ratio above this threshold; plant biomass may have declined at the highest applied fertilizer amount. According to Michaelis-Menten equation fit, maximal asymptote for biomass mean reaches 7.18 g (s.e.: 0.55 g), and half that size is reached at 0.73 g (s.e.: 0.23 g) of fertilizer.

Effect of integration on plant growth

Daughter plants connected with mothers generally accumulated more biomass than severed ones. In the low nutrient level, the connection increased daughter biomass by 0.013 g (95% c.i.: 0.009 to 0.017). Contrary to the expectations, the effect of connection was more pronounced when daughter plants grew in the high nutrient level with the additional increase in connected plants by 0.043 g (95% c.i.: 0.036 to 0.050) apart from the increase given by the high nutrient level itself (0.161 g, 95% c.i.: 0.157 to 0.164; Table 1, Fig. 4). The effect of connection on daughter biomass was not significantly influenced by the nutrient level of mother plants. Similarly, the importance of connection for daughter plants, estimated as the explained variation, was higher in the high nutrient level of daughter plants. With respect to nutrient level of mothers, no clear pattern of variation in daughter biomass explained by integration appeared (Table 2).

Connection increased the root/shoot ratio of daughter plants (1.184 times, 95% c.i.: 1.052 to 1.333), irrespective of nutrient level of either daughter or mother plants (Table 1, Fig. 4).

In mother plants, there was no significant effect of connection on either biomass or root/shoot ratio (P_F>0.1 for the main effect of connection and all its interactions with other terms; see Table 3).

Relative biomass allocation of whole pairs (i.e., allocation at the fragment level) to daughter plants was higher when daughters were grown in the high nutrient level (2.075 times, 95% c.i.: 1.584 to 2.718). In addition to the effect of a nutrient level, connection generally increased biomass allocation to the daughter plants (increase in low nutrient level of mothers 1.770 times, 95% c.i.: 1.348 to 2.325). Contrary to expectations, the positive effect of connection decreased with increasing nutrient level of mothers: The difference in allocation to daughters between connected and severed pairs was the biggest at the low nutrient level of mothers and declined in pairs that involved mothers at medium and high nutrient levels (the difference at any nutrient level decreased to 59.1 % [95% c.i.: 42.2 to 82.7] of the previous lower nutrient level; see Table 4, Fig. 5).

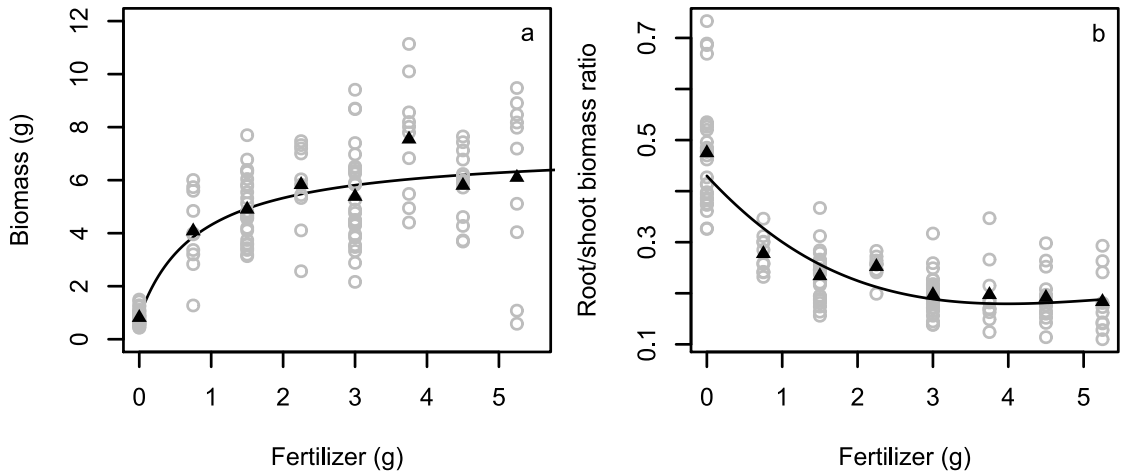


Figure 3 *Growth response of Agrostis stolonifera to fertilizer gradient in terms of (a) final biomass and (b) root/shoot ratio. Triangles show biomass means for plants in a given fertilizer dosage.*

Table 1 *Results of the linear models for the effect of initial size, nutrient level of daughter and mother plants and connection on biomass and root/shoot ratio of daughter plants. Significant effects (P_F < 0.05) are marked in bold. Sum of squares type I was used.*

Source of variance		Biomass of daughters			Root/shoot ratio of daughters		
	d.f.	Sum Sq	*F*	P_F	Sum Sq	*F*	P_F
Initial size	1	**10.81**	**296.7**	**< 0.001**	**0.81**	**28.4**	**< 0.001**
Nutrient level of daughters (D)	1	**3.74**	**102.5**	**< 0.001**	**3.68**	**129.4**	**< 0.001**
Nutrient level of mothers (M)	2	**2.18**	**29.9**	**< 0.001**	0.01	0.1	0.909
Connection (C)	1	**1.87**	**51.2**	**< 0.001**	**0.22**	**7.7**	**0.006**
D x M	2	0.25	3.5	0.034	0.02	0.4	0.695
D x C	1	**0.43**	**11.8**	**0.001**	0.01	0.5	0.483
M x C	2	0.01	0.1	0.865	0.05	0.8	0.454
D x M x C	2	0.06	0.9	0.419	0.10	1.8	0.163
Residuals	150	5.47			4.27		

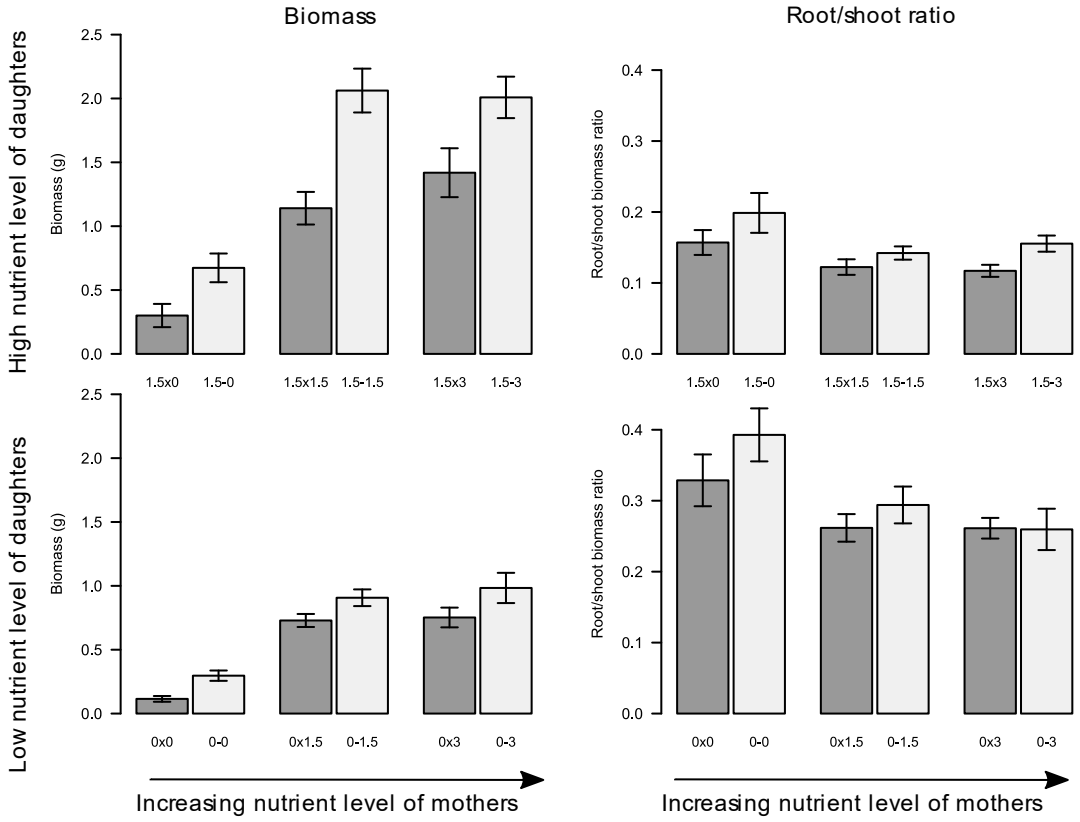


Figure 4 *Effect of connection on (a) biomass and (b) root/shoot ratio of daughter plants (means +- s.e.). Light grey bars are for the connected plants and dark grey bars are for the control plants. In the treatment code, the first number indicates nutrient level of daughters, x stays for control (severed) treatment, and - depicts connected ramets. The last number indicates the nutrient level of mothers.*

Table 2 *Variation in daughter biomass explained by connection in each combination of nutrient levels (in percent).*

	Mothers		
Daughters	Low nutrients	Medium nutrients	High nutrients
Low nutrients	11.3%	12.0%	8.3%
High nutrients	22.9%	46.6%	17.9%

Table 3 *Results of the linear models for the effect of initial size, nutrient level of daughter and mother plants and connection on biomass and root/shoot ratio of mother plants. Significant effects ($P_F < 0.05$) are marked in bold. Sum of squares type I was used.*

Source of variance		Biomass of mothers			Root/shoot ratio of mothers		
	d.f.	Sum Sq	*F*	*PF*	Sum Sq	*F*	*PF*
Initial size	1	**19.50**	**1380.18**	**< 0.001**	**2.62**	**1380.18**	**< 0.001**
Nutrient level of M	2	**5.41**	**191.37**	**< 0.001**	**1.27**	**191.37**	**< 0.001**
Nutrient level of D	1	0.00	0.22	0.640	0.00	0.22	0.696
Connection (C)	1	0.01	0.50	0.482	0.00	0.50	0.540
M x D	2	0.00	0.17	0.842	0.04	0.17	0.187
M x C	2	0.01	0.27	0.764	0.02	0.27	0.376
D x C	1	0.03	2.14	0.145	0.00	2.14	0.846
M x D x C	2	0.00	0.08	0.920	0.02	0.08	0.390
Residuals	153	2.16			1.72		

Table 4 *Results of the linear model for the effect of initial size of daughter and mother plants, nutrient level of daughter and mother plants and connection on daughter/mother biomass ratio. Significant effects ($P_F < 0.05$) are marked in bold. Sum of squares type I was used.*

Source of variance	Daughter/mother biomass ratio			
	d.f.	Sum Sq	*F*	P_F
Initial size of daughters	1	1.29	3.2	0.074
Initial size of mothers	1	**10.34**	**26.1**	**< 0.001**
Nutrient level of daughters (ND)	1	**13.74**	**34.7**	**< 0.001**
Nutrient level of mothers (NM)	2	0.47	0.6	0.554
Connection (C)	1	**20.78**	**52.5**	**< 0.001**
ND x NM	2	0.07	0.1	0.916
ND x C	1	0.90	2.3	0.134
NM x C	2	**4.89**	**6.2**	**0.003**
ND x NM x C	2	0.37	0.5	0.631
Residuals	149	58.98		

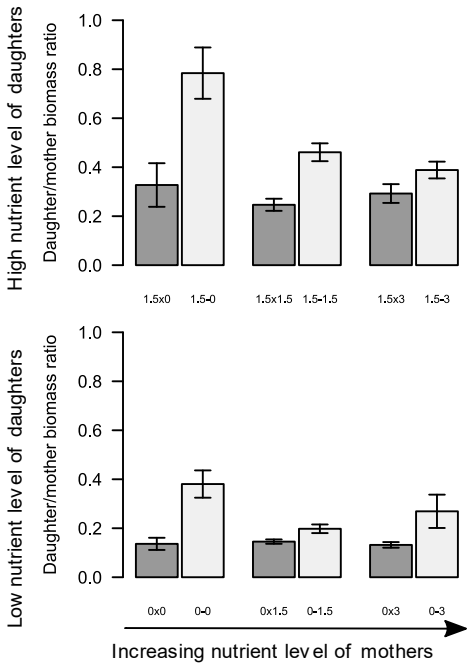


Figure 5 *Effect of connection on daughter/mother biomass ratio. Light grey bars are for the connected plants, and dark grey bars are for the control plants. In the treatment code, the first number indicates nutrient level of daughters, x stays for control (severed) treatment, and - depicts connected ramets. The last number indicates the nutrient level of mothers.*

Responses in clonal architecture of daughter plants

The number of stolons and maximum stolon length in daughters generally increased with their aboveground biomass (Fig. 6, Table 5). At the same time, the number of stolons was significantly higher in plants grown in the high nutrient level (1.276 times, 95% c.i.: 1.167 to 1.395), and connection did not significantly affect the number of stolons.

On the other hand, the maximum stolon length was significantly bigger in the low nutrient level than in the high nutrient level (by 0.088, s.e: 0.032 at the log-log scale), and its increase with plant biomass was significantly steeper for severed plants: Stolon length increase with the aboveground biomass was 0.298 (s.e.: 0.042; at the log-log scale) in connected plants, whereas for the severed plants, the slope increased by 0.122 (s.e.: 0:056; at the log-log scale). This means that the positive effect of connection on maximum stolon length was present only in small daughters, whereas the difference diminished in big daughters (Fig. 6).

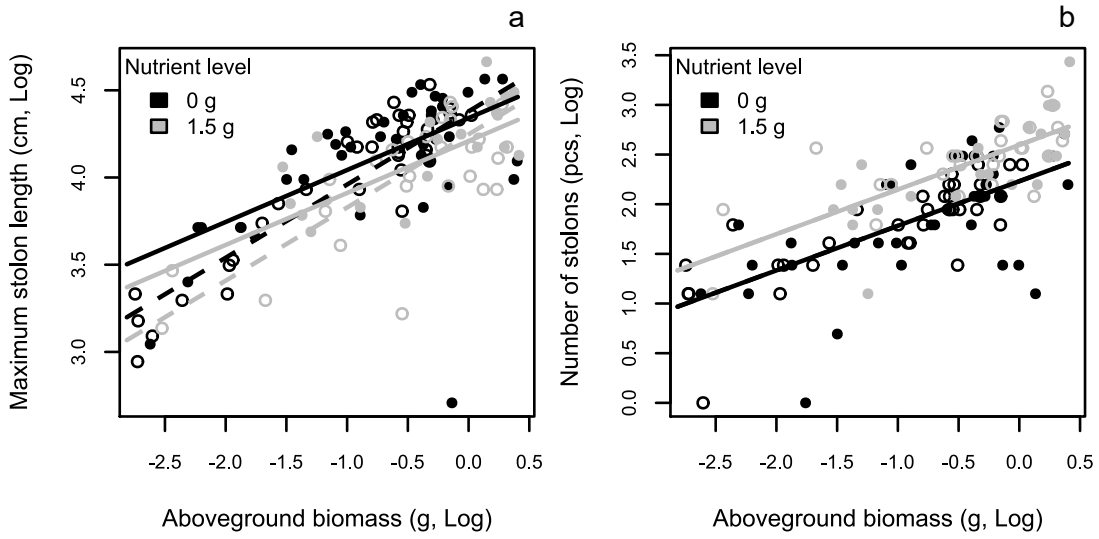


Figure 6 *Changes in (a) maximum stolon length and (b) number of stolons of daughter plants in response to their aboveground biomass, nutrient level and connection. Solid lines and full points depict the connected treatment, and dashed lines and empty points depict the control treatment. The nutrient levels of daughter plants are marked by different colours. Lines illustrate the significant effects (P_F<0.05, Table 4)*

Table 5 *Results of ANCOVAs for the effect of aboveground biomass, nutrient level of daughter plants and connection on maximum stolon length and number of stolons. Significant effects (P_F < 0.05) are marked in bold. Sum of squares type I was used.*

Source of variance		Maximum stolon length			Number of stolons		
	d.f.	Sum Sq	*F*	P_F	Sum Sq	*F*	P_F
Aboveground biomass (B)	1	**9.84**	**157.3354**	**< 0.001**	**21.01**	**154.9815**	**< 0.001**
Nutrient level of daughters (D)	1	**0.49**	**7.8957**	**0.006**	**3.78**	**27.8486**	**< 0.001**
Connection (C)	1	0.07	1.1535	0.285	0.13	0.9712	0.326
B:D	1	0.03	0.518	0.473	0.01	0.0423	0.837
B:C	1	**0.31**	**4.9567**	**0.028**	0.01	0.0612	0.805
D:C	1	0.20	3.2629	0.073	0.03	0.2137	0.645
B:D:C	1	0.07	1.0526	0.307	0.09	0.6295	0.429
Residuals	119	7.44			16.14		

Discussion

Effects of clonal integration on ramet performance and biomass allocation

We expected higher benefits and lower costs of integration in lower nutrient levels of daughters and higher nutrient levels of mothers. Instead, benefits from integration were more pronounced for daughters in the high nutrient levels, while there was no visible effect of integration on mothers in any treatment. At the same time, the relative allocation of whole clonal fragments to integrated daughters decreased with increasing nutrient levels of mothers, resulting in the highest investment to daughters growing in relatively rich conditions compared to mothers (i.e., in the same or higher nutrient level).

The nearly constant biomass ratio of severed daughter and mother plants suggests that this pattern was not caused simply by the greater biomass of mothers in higher nutrient levels. These findings contrast with our expectations as well as with previous experimental studies (Zhang et al. 2008; Wang et al. 2011), although the estimated shape of biomass increase in response to fertilizer clearly shows that the experiment was run in the parameter space well covered by the theory.

Integration with mother plants increased performance of daughters in all treatments, so the assumption of resource translocation being directed towards daughter plants was met. In addition, the positive effect of clonal integration in homogeneous conditions aligns with our predictions and supports the hypothesis of Dong et al. (2015) that for ramets differing in uptake capacity, clonal integration is also beneficial in homogeneous environments.

Furthermore, integration enhanced allocation to roots in daughter plants regardless of their nutrient level, while it had no effect on the root/shoot ratio of mother plants. This indicates increased demand of integrated daughter plants for nutrients, which is in contrast to the expected lower allocation to roots in integrated daughters growing in nutrient-poor conditions due to nutrient support from their mothers. In terms of division of labour in clonal plants, this indicates specialisation of daughters for nutrient uptake and subsequent export to mothers, even though some of the daughters grew in pure sand. The lack of detectable changes in biomass allocation of mother plants in response to integration may be caused by their bigger size and thus sufficient uptake ability to cover demands of the daughters.

Overall, the results may indicate that mother plants of *A. stolonifera* preferentially support daughters growing in relatively rich conditions, while they invest little into daughters at relatively poor sites. Such behaviour can be adaptive for preferential occupation of favourable patches by a clonal fragment while reducing costs of supporting ramets with low potential. Preferential allocation to ramets in nutrient-rich conditions was reported also from *Buchloe dactyloides*, in which heterogeneity in nutrients enhanced performance of nutrient-rich ramets and even suppressed performance of nutrient-poor ramets compared to homogenously rich or poor conditions (Sun et al. 2011). In addition, resource translocation may potentially reverse later in development, with daughter ramets eventually exporting resources to mothers (Marshall 1990). The enhanced allocation to roots in integrated daughters could be an indication of starting reverse translocation of nutrients from daughters to mothers.

An alternative explanation of the results may be that translocation of photosynthates rather than nutrients prevails between ramets of *Agrostis stolonifera* in the beginning of daughter plant development. Daughter plants in low nutrient levels thus could not benefit from such support because they are limited by nutrients, whereas daughters in high nutrient levels could utilise photosynthates better. In this case, increased allocation of integrated daughters to roots could be their response to abundant photosynthates. However, this explanation does not clarify the decrease of relative allocation

into integrated daughters with increasing nutrient levels of mothers. In an experiment investigating mineral nutrient interdependence of *A. stolonifera* ramets, the ramets appeared to become highly independent of the rest of clonal fragment after rooting (Marshall and Anderson-Taylor 1992), although translocation of phosphorus through clonal fragments has been previously shown in *Agrostis stolonifera* (Anderson-Taylor 1982). No information about carbon translocation in the species is available. Although several studies demonstrated translocation of a single type of resource directly by isotope tracing (Jonsdottir and Callaghan 1990; de Kroon et al. 1998; Xu et al. 2010), a comparison of the capacity to translocate different types of resources within clonal plant species is lacking (but see van Kleunen and Stuefer 1999). We thus cannot determine whether predominant translocation of one type of resource is expectable. Because the daughter plants originated from well-developed stolons, they had shoots from the beginning of their development. Therefore, carbon translocation from the mother plants was not inevitable for their growth.

The experiment has some restrictions that limit extrapolation of the results. First, it was done with a single clone of a single clonal species. Further data from experiments on mother and daughter ramets of other clonal species would reveal if the observed pattern is a rule or an exception. Second, the experiment lasted only 5 weeks, although connection between ramets in nature persists much longer. On the other hand, *A. stolonifera* is a really fast growing species, and the daughters were quite big (11.8 shoots per plant on the average) and potentially self-sustaining by the time of the harvest, even though they were still smaller than their mothers. Although some other effects of integration could be visible after a longer experimental period, the experiment shows the effect of integration in the relative beginning of daughter ramet development, when the size and uptake capacity of mothers and daughters are uneven.

Architectural responses

Daughters in low nutrient levels invested proportionally more into exploration of space by producing fewer stolons of greater maximal length than the nutrient-rich daughters of the same size. These responses to fertilization are in accordance with the concept of foraging in clonal plants (Sutherland and Stillman 1988; de Kroon and Schieving 1990) and our predictions.

Agrostis stolonifera has been previously shown to change mean length of stolons and internodes as well as stolon number in response to different light supplies (Dong and Pierdominici 1995), but architectural responses to nutrient availability have not been studied yet. Variation in maximum stolon length and the number of stolons has been found in *A. stolonifera* originating from different habitats and grown in a common garden experiment (Kik et al. 1990), indicating the ecological relevance of these traits. Plants originally from nutrient-poor sand dunes and highly competitive meadows had few relatively long stolons, which may be an adaptation to increased foraging in these habitats (Kik et al. 1990).

Although clonal foraging for nutrients seems to be less common than foraging for light (Dong and de Kroon 1994; de Kroon and Hutchings 1995), changes in clonal architecture in response to nutrient availability were found also in *Glechoma hederacea* (Slade and Hutchings 1987), *Brachypodium pinnatum* (de Kroon and Knops 1990), *Trientalis europaea* (Dong et al. 1997), *Halerpestes ruthenica* (Yu and Dong 2003), *Potentilla reptans* and *Potentilla anserina* (Louâpre et al. 2012). In *Scirpus olneyi*, a clonal plant forming short and long type of rhizomes, long rhizomes were produced irrespective of nutrient availability, whereas production of short rhizomes was promoted in rich conditions (Ikegami et al. 2007). Although internode length rather than maximum stolon length is often used as a measure of foraging activity (e.g., de Kroon & Hutchings 1995), we consider the maximum stolon length as a good approximation of foraging radius of plants.

Interestingly, integration with mother plants increased maximum stolon length in daughters, but only when the daughters were small. Similarly, integration enhanced foraging response in *Ranunculus reptans* grown in heterogeneous light supply (van Kleunen et al. 2000). Integration thus may affect performance of clonal plants not only by means of resource translocation, but also by modulating architecture of further clonal growth and foraging ability of plants.

Clonal growth of *A. stolonifera* seems to be well described by de Kroon and Schieving's (1990) model. The species is not a very strong competitor, so according to the model, the length of its stolons should vary according to resource supply, which we indeed found. The stolons were relatively longer and less numerous in nutrient-poor conditions, so *A. stolonifera* may be classified as a foraging species. However, when a clonal fragment was strongly limited (isolated and small), the strong limitation did not force the ramet to produce even longer foraging stolons, but to reduce investment in these stolons. Overall, this suggests the strong role of physiological integration in the strategy decision process: Even though currently equally small as severed ramets, integrated ramets invested more energy into exploration of space.

Conclusions

In sum, our results suggest that benefits of resource sharing need not increase with increasing contrast in resource availability of interconnected ramets when the ramets differ in size. Instead, benefits of clonal integration for young ramets growing in resource-rich conditions can exceed benefits for resource-poor ramets. We proposed two possible explanations of the observed patterns: (i) preferential support of daughter ramets at resource-rich patches to occupy favourable patches and (ii) translocation of photosynthates rather than nutrients between mother and daughter ramets of *Agrostis stolonifera*. Determination of types of resources translocated through clonal fragments of the species during establishment of young ramets may help explain the unexpected results.

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Electronic supplementary material

ESM 1 Plant biomass and architectural data reported in the paper. Plants excluded from all the analyses are not included in the dataset. Plants excluded only from the analyses of architectural responses are marked.
Electronic supplementary material is available online.

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Paper II

The direction of carbon and nitrogen fluxes between ramets of *Agrostis stolonifera* changes during ontogeny under simulated competition for light

Jana Duchoslavová[1], Jan Jansa[2]

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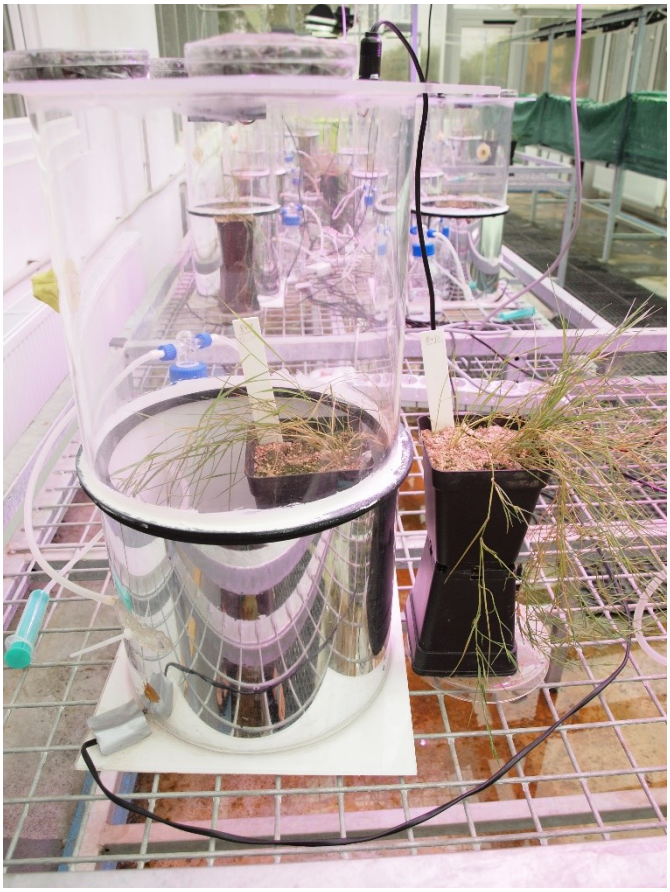
[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 43 Praha 2, Czech Republic

[2] Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Praha 4, Czech Republic



Abstract

Resource sharing is universal among connected ramets of clonal plants and is driven both by the developmental status of the ramets and the resource gradients. Above-ground competition forms spatial light gradients, but the role of resource sharing in such competition is unclear. We examined translocation of resources between mother and daughter ramets of *Agrostis stolonifera* under light heterogeneity throughout ramet ontogeny. We labelled ramets with ^{13}C and ^{15}N to estimate the bidirectional translocation of resources at three developmental stages of the daughters. In addition, we compared the final biomass of integrated and severed ramets in order to estimate the effect of integration on growth. Young developing daughters were supported by carbon, whereas nitrogen was only translocated towards daughters at the beginning of rooting, regardless of the light conditions. Shading of mothers was a major determinant of resource translocation between developed ramets, with carbon being preferentially moved to daughters from shaded mothers while nitrogen translocation was limited from daughters to shaded mothers. Surprisingly, the absolute amounts of translocated resources did not decline during development. Growth of daughters was enhanced by integration regardless of the shading. Overall, *A. stolonifera* maximizes the resource translocation pattern in order to enable it to spread from unfavourable habitats, rather than compensating for light heterogeneity among ramets.

Keywords: Carbon, clonal plant, development, light, nitrogen, ontogeny, physiological integration, ramet, stable isotopes, translocation.

Highlight

Carbon and nitrogen translocation between mother and daughter ramets of a clonal grass change during ramet ontogeny and are further affected primarily by the light conditions of the mother ramets.

Introduction

Clonal plants can form multiple ramets (i.e. potentially independent units with their own shoots and roots), which are interconnected by stolons or rhizomes. The connections between ramets enable plants to share resources and information through the integrated system (Marshall, 1990; Song *et al.*, 2013), which may provide them with an advantage over non-integrated plants that rely solely on the resources present in their patch, especially when resources are distributed heterogeneously (e.g. Friedman and Alpert, 1991). Resource translocation is particularly important for new, developing ramets, which are supported by water, mineral nutrients, and/or photosynthates from older and more developed ramets (Hartnett and Bazzaz, 1983; Pitelka and Ashmun, 1985; Marshall, 1990). Due to the parental support, new ramets can have an advantage over seedlings when they are growing in competitive or stressful conditions (Sarukhan and Harper, 1973). However, it has also been shown that mineral nutrients may be preferentially translocated from daughter to larger parental ramets to meet the higher nutrient demand of the latter (Pinno and Wilson, 2014). The initial resource translocation may decline as ramets develop and become self-sustainable in resource acquisition (Hartnett and Bazzaz, 1983). However, exchange of resources can be maintained even among developed ramets, and it can be induced or enhanced by heterogeneity in resource availability, local stress, or differential resource needs of the ramets (Pitelka and Ashmun, 1985; Song *et al.*, 2013). Accordingly, benefits of clonal integration have been shown to increase with increasing heterogeneity in the availability of water (Pennings and Callaway, 2000), mineral nutrients (Alpert, 1991, 1996; Birch and Hutchings, 1994), or light (Stuefer *et al.*, 1994; Xu *et al.*, 2010).

Heterogeneity in both light and below-ground resources can be due to the abiotic environment or generated by plant interactions themselves (Chazdon and Pearcy, 1991; Skálová *et al.*, 1999). Vegetation may form strong gradients in light availability, especially when below-ground resources are abundant, and competition for light then becomes the dominant factor affecting plant growth. Therefore, the ability to cope with light heterogeneity may be essential for plants in such environments. In addition, smaller plants are disproportionally handicapped because competition for light is size-asymmetric (Weiner, 1990). Integration of ramets may allow clonal plants to cope with light heterogeneity by support of (temporarily) shaded ramets and thereby partially compensate for the asymmetry of light competition (de Kroon *et al.*, 1992). Indeed, benefits of clonal integration for shaded ramets have been demonstrated by growth experiments (e.g. Hartnett and Bazzaz, 1983; Stuefer *et al.*, 1994; Xu *et al.*, 2012), although in some cases, the parental support may cease when shading lasts too long (Hartnett and Bazzaz, 1983). In addition, the spread from open patches to neighbourhoods of other species is facilitated by clonal integration in some species (Roiloa *et al.*, 2010; Xiao *et al.*, 2011). In others, however, clonal integration mainly enhances the exploration of open space and the quick expansion into unvegetated patches (Pennings and Callaway, 2000). The effect of integration on the growth of the whole clonal plant under competition also differs among

species (Peltzer, 2002; Pauliukonis and Gough, 2004; Wang *et al.*, 2016). The role of clonal integration in light competition is, therefore, still far from clear, although it has recently gained increasing attention, especially in connection with invasive species (e.g. Yu *et al.*, 2009; You *et al.*, 2014; Wang *et al.*, 2016).

Shading has been shown to affect carbon translocation between ramets in several plant species (Qureshi and Spanner, 1973; Pitelka and Ashmun, 1985). For example, in an experiment simulating clonal spread from a bare habitat (full sun) to vegetation shade (85% shade cloth), an enhanced transport of photosynthates from unshaded parent ramets to continuously shaded daughter ramets was observed in *Alternanthera philoxeroides*, whereas in *Phyla canescens* carbon import did not differ between shaded and unshaded daughter ramets (Xu *et al.*, 2010). Moreover, differences in growth of integrated and severed ramets of the two species corresponded to the observed differences in translocation (Xu *et al.*, 2010, 2012). In contrast to carbon, translocation of mineral nutrients in response to light gradients has to date been rarely studied, although differential availability of light may induce an imbalanced in needs for nutrients in different ramets, even though nutrients in the substrate may be distributed homogeneously. Indeed, the results of Saitoh *et al.* (2006) indicated that nitrogen translocation from shaded to unshaded ramets could be enhanced due to higher sink activity of developing unshaded leaves. Moreover, the supply of shaded ramets with photosynthates can increase their ability to assimilate nitrogen from the soil (Chen *et al.*, 2015).

Patterns of resource translocation are likely to change markedly during ramet development. However, so far research has usually focused on a single resource type at a single point in time in ramet ontogeny (but see Alpert *et al.*, 2002; Luo *et al.*, 2014). Furthermore, the assessment of both the physiological and ecological relevance of nutrient flows is possible only if direct labelling of the specific resource is carried out so as to trace the transport in either direction. Combining the results of independent experiments is complicated by differing assimilation capacities and physiological status of experimental plants. Surprisingly, only a few studies have so far examined the transport of labelled resources in both directions between the parent and daughter ramets (but see Alpert, 1996; Pinno and Wilson, 2014). Thus, a coherent picture of net translocation of both carbon and nutrient resources among developing ramets in a heterogeneous environment is still lacking.

We addressed this gap by simultaneous examination of nitrogen and carbon flow between mother and daughter ramets during the development of the daughter ramet. We used pairs of mother and daughter ramets of a stoloniferous grass, *Agrostis stolonifera*, to study the exchange of carbon and nitrogen under heterogeneous light conditions, simulating above-ground competition through generating light gradients. We estimated bidirectional translocation of carbon and nitrogen between ramets at three developmental stages: at the very beginning of daughter rooting, during the initial daughter

development, and finally when the size of the daughter reached that of the mother. At the same time, we measured the effect of clonal integration on plant growth under the same conditions.

We hypothesized that, in general, initial translocation of both resources towards daughters would decline with time as the daughters develop their own assimilation structures and become functionally independent of the mother. Furthermore, we expected a relatively strong effect of light gradient on the carbon flow between the ramets, with translocation directed preferentially to shaded daughter ramets, and reverse net carbon translocation from developed daughters to shaded mothers. With respect to nitrogen, we hypothesized that its translocation from shaded to unshaded ramets could be enhanced because of differential growth rates of shaded and unshaded ramets. In addition, daughter ramets with sufficiently developed roots may provide mother ramets with nitrogen from the newly occupied patch.

Materials and methods

Growth habit of Agrostis stolonifera

Agrostis stolonifera is a perennial stoloniferous grass, common in mesic and wet grasslands and river banks (Kik *et al.*, 1990; Kubát *et al.*, 2002). Clonal reproduction prevails over propagation through seeds in this species. Developed ramets are composed of multiple tillers, which may bend groundwards and develop into stolons, forming leaves and roots at some of their nodes. Connections among ramets persist for the whole vegetative season and can overwinter. The plant material used in this experiment originated from a single genotype collected in a field and grown in an experimental garden since 2010. None of the plants flowered during the experiment.

Initial cultivation

Tillers of source plants were cut and placed on wet sand to initiate rooting in mid-March 2016. Individual ramets (i.e. single nodes with developed leaves and roots) were then separated and planted in 1-l pots with a mixture of washed sand and slow-release fertilizer (3 g per pot, Substral Osmocote Grass, www.substral.cz; gravimetric nutrient content: N, 23%; P, 5%; K, 10%; Mg, 2%; S 9%) at the beginning of May 2016. These ramets are referred to as the mother ramets. The pots were positioned in a greenhouse equipped with supplemental lighting [400 W metal halide lamps providing a minimum of 200 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), extending the daylight period to 14 h] and watered three times a day with tap water. Dead ramets were replaced by new ones until the end of May 2016.

A shading cloth was installed 3 d before the establishment of daughter ramets. Plants were shaded from the top and all sides by the shading cloth combined with strips of green foil (3-cm wide, with 3-cm gaps in between, LeeFilters Fern Green 122, www.leefilters.com) to simulate changes in both light quantity and quality caused by above-ground competition (80% PAR reduction and 30% reduction of

the red to far-red ratio). Shading also reduced the air temperature (see Supplementary Data Fig. S1 at *JXB* online for details).

Experimental design

Formation of daughter ramets was initiated over 3 d between 13–15 June 2016 by placing the longest stolon of each mother ramet in an adjacent vacant pot. For logistical reasons, each labelling and harvest campaign was also conducted over three subsequent days, so that the same intervals between the daughter ramet initialization and harvest were maintained for all plants for each age cohort of daughter ramets. Therefore, plant units (ramet pairs) processed at each harvest campaign (i.e. for a given ontogeny stage) were divided into three time-blocks with 1-d differences in initialization/harvest. Treatments were represented evenly among the blocks. A factorial design with mothers and daughters grown either in full light or under green shade was used (Fig. 1a).

Bidirectional translocation of carbon and nitrogen between mothers and daughters was examined by stable-isotope labelling (using ^{13}C and ^{15}N) at three developmental stages of the daughters: at the time of rooting (i.e. labelling took place immediately after daughter ramet initialization), 2 weeks after rooting, and 8 weeks after rooting (Fig. 1b). Four replicates (ramet pairs) were used for each combination of shading treatment, direction of translocation, and time of labelling.

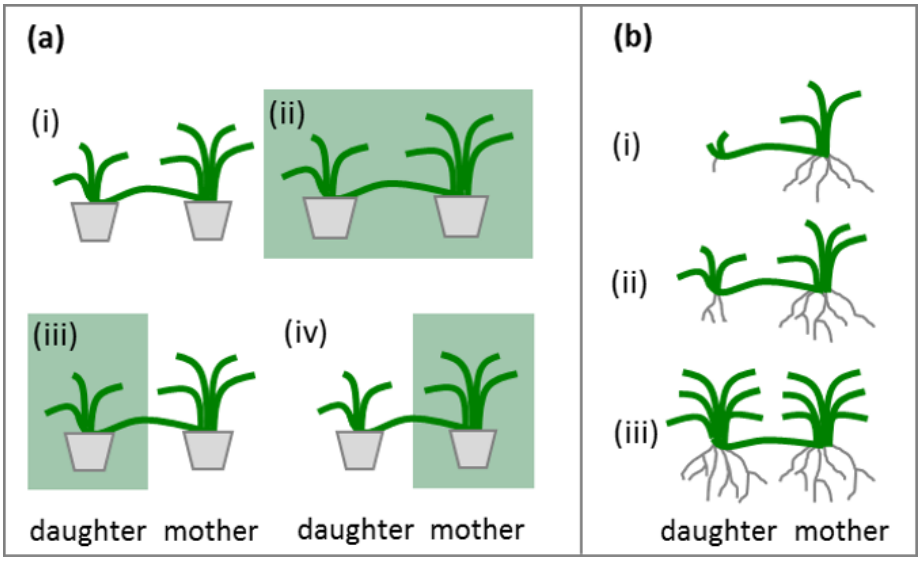


Figure 1 *Design of the experiment. (a) Four shading treatments were used: (i) both ramets in full light, (ii) both ramets under green shade, (iii) daughter ramet shaded, or (iv) mother ramet shaded. (b) Schematic representation of ramet size at the time of harvests: (i) first (0 weeks), (ii) second (2 weeks), and (iii) final (8 weeks after daughter establishment).*

Stable-isotope labelling

Plants were pulse-labelled simultaneously with nitrogen (^{15}N) and carbon (^{13}C) according to a protocol tested in a pilot experiment (J. Duchoslavová and J. Jansa, unpublished results). Each labelling started at approximately 08.00 h (the photoperiod began at 06.00 h). Nitrogen was applied directly to pots by

a syringe in the form of double-labelled ammonium nitrate (99 atom% ^{15}N, 15 mg per pot). Carbon was applied in the form of $^{13}CO_2$ (see Supplementary Data Figs S2, S3 for photographs), as follows. Labelled ramets (single pots) were enclosed in Plexiglass chambers equipped with a fan to mix the inner atmosphere, and $^{13}CO_2$ was released inside the chambers by injecting 20 ml of phosphoric acid (20%, w:v) into a vial with ^{13}C-enriched sodium carbonate (99% atom% ^{13}C, 0.3 g per pot, calculated initial $^{13}CO_2$ concentration inside chambers reaching 3100 ppm). The ramets were allowed to assimilate the labelled carbon for 2 h, and then the remaining CO_2 in the atmosphere was scrubbed by circulating the air through NaOH solution (0.1 M, 200 ml) before opening the chamber. The labelling took place in full sunlight supplemented by additional light from diode lamps (LumiGrow Pro Series Pro 325 LED Lighting Systems, photosynthetic photon flux density 333 μmol m^{-2} s^{-1} at a distance of 1 m), and the plants were returned to the experimental shading conditions immediately after the labelling period. The mean light intensity inside the labelling chambers reached between 180–600 μmol m^{-2} s^{-1} PAR, depending on the day (data not shown).

Labelled ramet pairs were always harvested exactly 2 d after labelling. Mother and daughter ramets were separated; the roots were washed and separated from the shoots. The plant material was then dried (60 °C for 48 h), weighed, and milled to a fine powder using a ball mill (MM200, Retsch, Haan, Germany) before the elemental and isotopic analyses. The N and C concentrations and isotopic compositions of the two elements were measured using an elemental analyser (Flash EA 2000) coupled with an isotope-ratio mass-spectrometer (Delta V Advantage, ThermoFisher Scientific, Waltham, MA, USA).

To calculate the amount of ^{13}C and ^{15}N originating from pulse-labelling (i.e. excess ^{13}C or ^{15}N), *F*-ratios were calculated as $R_S/(R_S+1)$, where R_S is the molar isotope ratio in a sample ($^{15}N/^{14}N$ or $^{13}C/^{12}C$). The amount of total carbon or nitrogen (*C*, in moles) was then calculated as:

$$C = (W_{dry} \times B)/[a \times (F+b) \times (F-1)] \quad (1)$$

where W_{dry} is dry weight of a sample, *B* is molar concentration of carbon or nitrogen in a sample, a is 12 for carbon and 14 for nitrogen, b is 13 for carbon and 15 for nitrogen, and *F* is the *F*-ratio of the respective element in a sample. The amount of ^{13}C and ^{15}N originating from pulse-labelling (*E*, in moles) was finally calculated as:

$$E = (F_s - F_U) \times C \quad (2)$$

where F_S is the *F*-ratio of a sample and F_U is the mean *F*-ratio of four unlabelled control ramet pairs for each harvest. The amount of ^{13}C and ^{15}N originating from pulse-labelling that was found in unlabelled ramets (roots and shoots combined) is hereafter referred to as the amount of translocated ^{13}C and ^{15}N.

Severed ramet pairs

To estimate the effect of integration on ramet growth (biomass), additional ramet pairs were cultivated in the same conditions as the plants used for isotopic labelling (with eight replicates per shading treatment). In these plants, the connections between ramets were severed 1 week after establishment of the daughter ramets. These additional plants were harvested at the time of the final harvest (i.e. 8 weeks after daughter ramet initialization) in the same way as the labelled plants, but they were not analysed for their elemental and isotopic composition.

Data analyses

All statistical analyses were performed in the R statistical environment (version 3.3.2, www.r-project.org).

The effects of shading, time since daughter establishment, and direction of translocation on the amount of translocated label and on the proportion of the label exported from a labelled ramet were tested by separate linear models for carbon and nitrogen. Shading treatment was included in the models as shading of mothers and shading of daughters (i.e. two two-level factors). Time was included as a three-level factor, with the first harvest as a reference level. The proportion of exported label was calculated as the ratio of label amount in an unlabelled ramet and as the total label amount in a ramet pair. The proportion of exported labels as well as the absolute amount of translocated carbon and nitrogen were log-transformed to meet model assumptions. Effect estimates based on treatment contrasts and corresponding t and P_t values were used for interpretation of the models.

The effects of integration and shading on ramet growth were tested by separate linear models for mother and daughter ramets. The initial size of ramets, measured as total length of their tillers at the time of daughter establishment, was included as a covariate. The dry biomasses of integrated and severed ramets at the time of the final harvest were used as response variables, and they were log-transformed to meet model assumptions.

Results

Ramet size

At the time of the first harvest, mothers had on average 2.4 times higher dry biomass than daughters (Table 1), and the single, emerging roots of daughters were on average 7 mm long. At the time of the second harvest, mother ramets in full-sun and full-shade treatments had 2.1 times higher dry biomass than daughters (Table 1). When only daughters were shaded, mothers had 2.8 times higher dry biomass, and biomass of both ramets was similar when only mothers were shaded (Table 1). At the final harvest, the biomass of daughters in homogeneous treatments reached that of the mothers. When only one ramet was shaded, the ramets in the sun had 4.4–4.5 times higher biomass than the shaded ramets (Table 1).

Table 6 *Biomass of daughter and mother ramets at the times of the first, second and final harvest*

	Biomass of daughters (g)		**Biomass of mothers (g)**	
First harvest[1]	mean	SE	mean	SE
	0.07	0.01	0.17	0.02
Second harvest	mean	SE	mean	SE
Full light	0.47	0.07	1.01	0.12
Daughter shaded	0.32	0.05	0.65	0.14
Mother shaded	0.17	0.02	0.16	0.03
Full shade	0.15	0.03	0.32	0.08
Final harvest	mean	SE	mean	SE
Full light	6.91	0.53	6.07	0.52
Daughter shaded	1.12	0.18	5.11	0.74
Mother shaded	5.7	0.54	1.28	0.24
Full shade	0.35	0.06	0.43	0.09

[1]Biomass was not affected by the shading treatment due to its short duration at the first harvest.

Translocation of carbon

The amount of translocated carbon was significantly affected by the direction of translocation and shading of mother ramets. The effects of direction and shading changed significantly with time. Shading of daughters did not have a significant effect on carbon translocation (for *F*- and *P*-values see Table 2).

At the beginning of daughter establishment (first harvest), more carbon was translocated towards mothers than towards daughters, with no significant effect of shading (Fig. 2). Two weeks after daughter establishment, the translocation of carbon towards mothers declined, whereas the translocation towards daughters rose relative to the first harvest regardless of the light treatment (Fig. 2), resulting in net flow of carbon directed towards daughters (Fig. 3). Shading had no significant effect on carbon translocation at the second harvest. Eight weeks after daughter establishment, carbon translocation towards unshaded mothers was lower than at the first harvest, resulting in balanced carbon translocation in both directions (Fig. 2). However, when mothers were shaded, translocation towards mothers declined considerably, and net carbon flow was consequently directed towards daughters (Figs 2, 3).

The fraction of assimilated ^{13}C exported towards unlabelled ramets significantly decreased with time. Daughters initially exported proportionally more labelled carbon than mothers, but this difference was reversed and eventually diminished at the second and the final harvests, respectively. Furthermore, daughters initially exported a smaller proportion of the ^{13}C when shaded, whereas shading had no

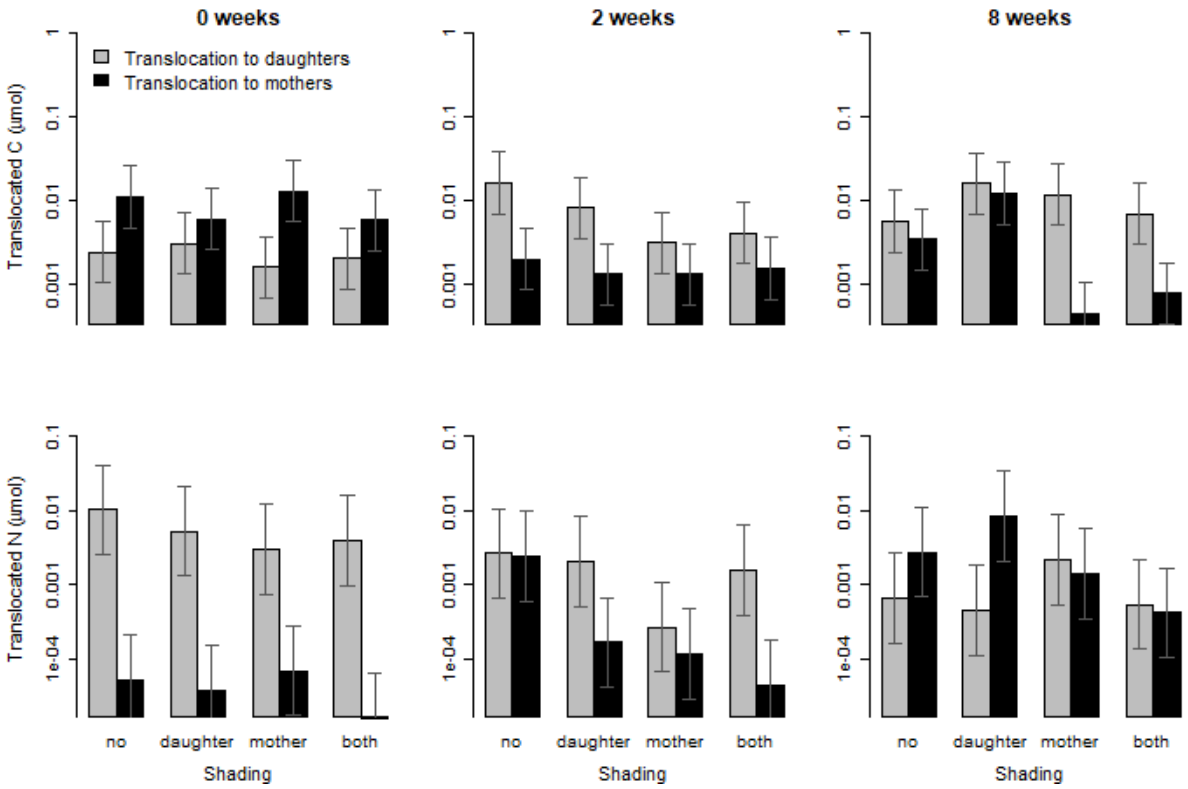


Figure 2 *Amounts of translocated 13C and 15N (means and 95% confidence intervals) from labelled mothers towards daughter ramets and from labelled daughters towards mother ramets under different shading treatments and at different times from the establishment of daughter ramets. Note that the y-axes are log scales.*

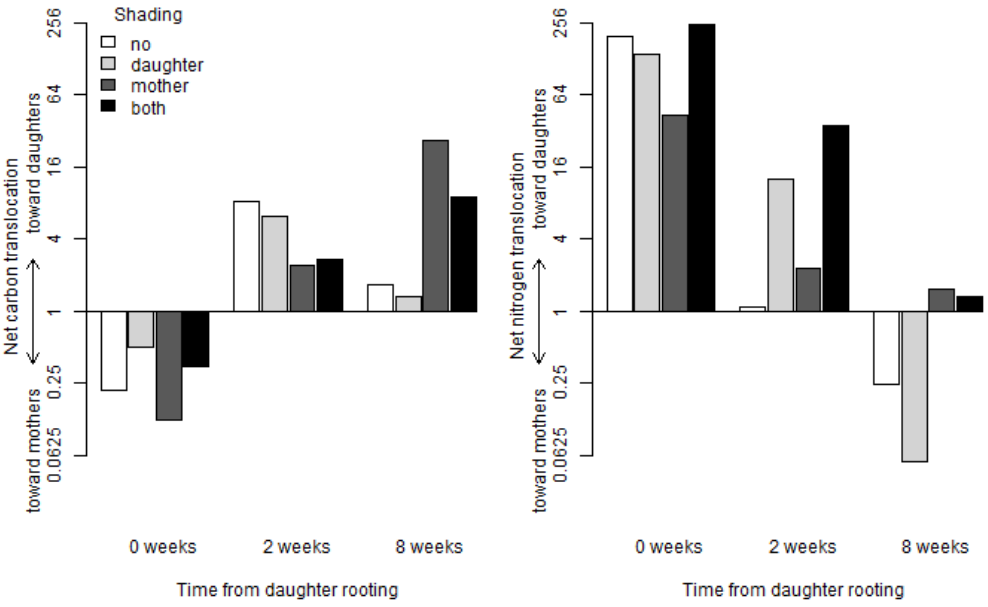


Figure 3 *Net carbon and nitrogen translocation between mother and daughter ramets expressed as the log ratio of translocated label towards daughters and towards mothers. Values above 1 indicate prevailing translocation towards daughters, whereas values below 1 indicate prevailing translocation towards mothers.*

Table 2. *ANOVA of linear models of the amount of ^{13}C and ^{15}N (log-transformed) translocated towards unlabelled ramets in response to direction of translocation, shading of mothers (M) and daughters (D), and time of labelling*

		Translocated ^{13}C			**Translocated ^{15}N**		
	d.f.	Sum Sq[1]	*F*	P_F	Sum Sq[1]	*F*	P_F
Direction	**1**	**7.37**	**10.17**	**0.002**	**87.03**	**44.12**	**<0.001**
M shaded	**1**	**11.15**	**15.38**	**<0.001**	**21.45**	**10.87**	**0.002**
D shaded	1	0.09	0.12	0.733	6.35	3.22	0.077
Time	2	2.13	1.47	0.236	19.34	4.90	0.010
Dir. x M shaded	1	0.47	0.64	0.425	5.67	2.88	0.094
Dir. x D shaded	1	0.03	0.04	0.841	4.13	2.09	0.152
M shaded x D shaded	1	0.14	0.19	0.664	0.00	0.00	0.964
Dir. x time	**2**	**43.38**	**29.92**	**<0.001**	**134.30**	**34.04**	**<0.001**
M shaded x time	2	4.36	3.01	0.056	7.35	1.86	0.163
D shaded x time	2	3.33	2.29	0.108	0.03	0.01	0.993
Dir. x M shaded x D shaded	1	0.01	0.02	0.897	2.29	1.16	0.285
Dir. x M shaded x time	**2**	**13.22**	**9.12**	**<0.001**	8.82	2.24	0.114
Dir. x D shaded x time	2	2.58	1.78	0.177	11.73	2.97	0.057
M shaded x D shaded x time	2	3.64	2.51	0.088	**12.45**	**3.16**	**0.049**
Dir. x M shaded x D shaded x time	2	0.46	0.32	0.730	0.81	0.21	0.815
Residuals	72	52.20			142.04		

effect on ^{13}C export from daughters at the second harvest, and shaded daughters exported a higher proportion of the label at the final harvest. At the time of the final harvest, daughters exported a markedly smaller proportion of labelled carbon towards shaded mothers than when the mothers were unshaded (Tables 3, 4).

Translocation of nitrogen

The amount of translocated nitrogen was significantly affected by the direction of translocation, the time from the establishment of daughter ramets, and the shading of mother ramets, of which the shading generally decreased nitrogen translocation. The effect of the direction changed significantly with time (see Table 2).

At the beginning of daughter establishment, the amount of nitrogen translocated towards daughters was higher than that of nitrogen translocated towards mothers, with the light treatment having no effect (Fig. 2). Two weeks after daughter establishment, translocation towards mothers rose to values roughly equivalent to translocation towards daughters (Fig. 2). Absolute translocation towards daughters did not significantly differ from that observed at the first harvest. There was no significant effect of shading on nitrogen translocation at the second harvest. Eight weeks after daughter establishment, nitrogen translocation towards mothers increased, whereas translocation towards daughters declined relative to the first harvest, resulting in slightly higher net translocation of nitrogen towards mothers than towards daughters (Figs 2, 3).

Table 3. *Fractions of assimilated ^{13}C and ^{15}N exported from labelled ramets towards unlabelled ramets*

	Exported carbon						**Exported nitrogen**					
First harvest	**from mothers**			**from daughters**			**from mothers**			**from daughters**		
	fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.	
Full light	**2.70%**	1.01	7.19	**19.27%**	7.23	51.32	**35.34%**	9.59	130.30	**25.26%**	6.85	93.14
Daughter shaded	**3.42%**	1.28	9.10	**7.53%**	2.83	20.05	**26.61%**	7.22	98.11	**15.50%**	4.20	57.13
Mother shaded	**2.55%**	0.96	6.79	**12.65%**	4.75	33.70	**30.02%**	8.14	110.65	**14.03%**	3.80	51.71
Full shade	**2.89%**	1.09	7.70	**3.84%**	1.44	10.22	**42.80%**	11.61	157.77	**13.77%**	3.73	50.75
Second harvest	**from mothers**			**from daughters**			**from mothers**			**from daughters**		
	fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.	
Full light	**2.91%**	1.09	7.76	**0.78%**	0.29	2.08	**2.39%**	0.65	8.80	**6.56%**	1.78	24.18
Daughter shaded	**2.31%**	0.87	6.16	**0.55%**	0.21	1.47	**3.25%**	0.88	11.99	**2.91%**	0.79	10.73
Mother shaded	**2.18%**	0.82	5.82	**0.67%**	0.25	1.80	**2.62%**	0.71	9.65	**0.76%**	0.21	2.81
Full shade	**2.04%**	0.76	5.42	**1.30%**	0.49	3.46	**8.54%**	2.32	31.47	**2.43%**	0.66	8.97
Final harvest	**from mothers**			**from daughters**			**from mothers**			**from daughters**		
	fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.		fitted	95% c.i.	
Full light	**0.54%**	0.20	1.44	**0.39%**	0.15	1.04	**0.31%**	0.08	1.13	**1.42%**	0.39	5.24
Daughter shaded	**1.23%**	0.46	3.27	**1.90%**	0.71	5.06	**0.20%**	0.06	0.75	**14.44%**	3.92	53.23
Mother shaded	**2.24%**	0.84	5.98	**0.04%**	0.02	0.11	**3.36%**	0.91	12.38	**0.59%**	0.16	2.16
Full shade	**4.20%**	1.58	11.18	**0.38%**	0.14	1.02	**3.22%**	0.87	11.86	**1.36%**	0.37	5.03

[a]Expected (untransformed) values of the variable in the model (using log-transformed values).
[b]Limits of 95% confidence intervals (CI).

Table 4 *ANOVA of linear models of the percentage of exported ^{13}C and ^{15}N (log-transformed) from labelled ramets in response to direction of translocation, shading of mothers (M) and daughters (D), and time of labelling*

		Exported ^{13}C			**Exported ^{15}N**		
	d.f.	Sum Sq[1]	*F*	P_F	Sum Sq[1]	*F*	P_F
Direction	**1**	**6.04**	**6.25**	**0.015**	0.23	0.13	0.717
M shaded	1	0.84	0.87	0.355	0.01	0.01	0.941
D shaded	1	2.05	2.12	0.149	2.83	1.65	0.203
Time	**2**	**64.24**	**33.25**	**<0.001**	**139.00**	**40.56**	**<0.001**
Dir. x M shaded	1	6.54	6.77	0.011	**27.33**	**15.95**	**<0.001**
Dir. x D shaded	1	0.04	0.04	0.838	0.59	0.35	0.559
M shaded x D shaded	1	0.27	0.28	0.597	1.34	0.78	0.379
Dir. x time	**2**	**34.05**	**17.62**	**<0.001**	9.97	2.91	0.061
M shaded x time	2	0.81	0.42	0.660	2.70	0.79	0.459
D shaded x time	**2**	**13.42**	**6.94**	**0.002**	2.65	0.77	0.466
Dir. x M shaded x D shaded	1	0.41	0.42	0.519	0.14	0.08	0.779
Dir. x M shaded x time	**2**	**15.70**	**8.12**	**0.001**	**14.21**	**4.15**	**0.020**
Dir. x D shaded x time	2	6.08	3.15	0.049	6.74	1.97	0.147
M shaded x D shaded x time	2	0.58	0.30	0.740	3.93	1.15	0.323
Dir. x M shaded x D shaded x time	2	0.33	0.17	0.843	2.16	0.63	0.535
Residuals	72	69.57			123.37		

[a]Sum of squares type I was used.
[b]Effects with $P<0.05$ are highlighted in bold.

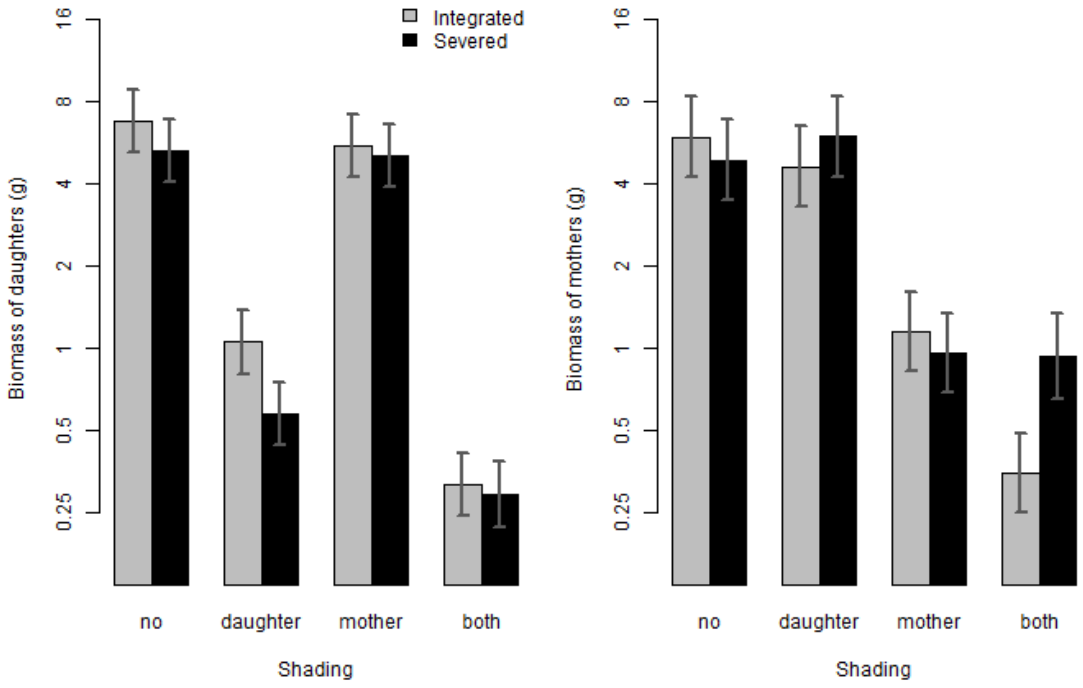


Figure 4 *Final dry biomass (log scale, means and 95% confidence intervals) of daughter and mother ramets with intact or severed connections under different shading treatments.*

Table 5 *ANOVA of linear models of final biomass of daughter and mother ramets (log-transformed) in response to shading of mothers (M), shading of daughters (D), and integration*

		Daughter ramet biomass			**Mother ramet biomass**		
	d.f.	Sum Sq[1]	*F*	P_F	Sum Sq[1]	*F*	P_F
Initial size	1	0.58	4.53	0.038	14.87	95.34	<0.001
M shaded	**1**	**3.56**	**27.99**	**<0.001**	**51.41**	**329.53**	**<0.001**
D shaded	**1**	**93.51**	**735.7**	**<0.001**	**1.13**	**7.26**	**0.009**
Integration	1	**1.24**	**9.77**	**0.003**	0.51	3.24	0.078
M shaded : D shaded	**1**	**2.74**	**21.54**	**<0.001**	**0.79**	**5.04**	**0.029**
M shaded : integration	1	0.43	3.39	0.071	0.44	2.83	0.098
D shaded : integration	**1**	0.39	3.07	0.085	**0.64**	**4.1**	**0.048**
M shaded : D shaded : integration	1	0.11	0.89	0.349	0.41	2.61	0.112
Residuals	54	6.86			8.43		

[a]Sum of squares type I was used.

The fraction of assimilated ^{15}N exported towards unlabelled ramets significantly decreased with time. Daughters exported a smaller proportion of labelled nitrogen towards shaded mothers, especially at the time of the final harvest. Correspondingly, shaded mothers exported proportionally more labelled nitrogen towards daughters at the time of the final harvest (Tables 3, 4).

Effect of integration on ramet growth

Shading had a significant effect on the growth of both daughter and mother ramets. In daughter ramets, integration had a positive effect on growth, with no significant interactions of integration and shading. In mother ramets, the effect of integration alone was not significant, but integration with shaded daughters had a marginally significant negative effect on the growth of mothers (Table 5, Fig. 4).

Discussion

Our results showed changes in the translocation of both above-ground (carbon) and below-ground (nitrogen) resources between mother and daughter ramets of *Agrostis stolonifera* during the course of daughter ramet development, as well as an effect of light gradients on resource translocation. Resource translocation in the initial stages of daughter ramet development (ontogeny) was generally in accordance with our expectation of initial resource translocation directed towards daughters. Nitrogen was translocated predominantly towards daughters at the very beginning of their establishment, and carbon translocation was directed towards the rapidly developing daughters 2 weeks after the beginning of their rooting (Fig. 3). Contrary to our predictions, absolute translocation of resources during early daughter ramet development was not affected by light availability to the different ramets. Overall, the magnitude of absolute resource flows did not decline with time as we originally expected, although individual ramets were obviously self-sustaining in terms of resource acquisition at the late

developmental stage, i.e. 8 weeks after daughter rooting (Fig. 2). The amounts exported from labelled ramets, however, accounted for smaller proportions of the total assimilated resources at later stages of development than at the beginning (Table 3). Light conditions significantly altered carbon translocation only at the late developmental stage (i.e. 8 weeks after daughter rooting) and, unexpectedly, the net translocation of carbon (Fig. 3) as well as the proportional export of assimilated nitrogen (Table 3) were directed from shaded mothers towards daughters, irrespective of daughter light conditions.

Photosynthates are translocated by the phloem, and their movement in the plant body is determined primarily by the activity of sinks and sources. Nitrogen as well as other phloem-mobile nutrients can be translocated by both the transpiration flow in the xylem and by the phloem (Marshall, 1990). Transport of resources thus has a rather complex nature, as plants can, for example, further modify the phloem flow by hormonal control (Alpert *et al.*, 2002).

Resource translocation during ramet development

Translocation of resources to emerging ramets as well as the crucial role of integration in early development of new ramets are well documented in the literature (e.g. Hartnett and Bazzaz, 1983; Alpert, 1996; Xu *et al.*, 2012), but it is not clear if resource gradients alter maternal support at this stage (Alpert and Mooney, 1986). We observed that carbon was translocated predominantly from establishing daughters towards mothers at the very beginning of daughter rooting (Fig. 3), which showed that the developed tillers that formed daughter ramets were self-reliant in terms of carbon assimilation and, at the same time, smaller tillers continuously being formed on the mother ramet functioned as a strong sink for carbon. At that time, the resource exchange between ramets probably still corresponded to the resource exchange among unrooted tillers of a single ramet, which function essentially as leaves. Initial carbon translocation may, however, be directed towards daughter ramets in other plant species with different ramet morphology (e.g. daughter ramets having smaller leaf area in comparison to mothers). For example, translocation of carbon from older leaves to unrooted daughter nodes was observed in white clover (Kemball and Marshall, 1995). Net carbon translocation in our study was not significantly affected by light gradients at the initial stage, although shaded daughters exported proportionally less carbon towards mothers (Table 3). In contrast, Alpert and Mooney (1986) reported that carbon translocation towards unrooted ramets of *Fragaria chiloensis* was induced by shading these ramets. The observed initial strong nitrogen translocation towards almost-unrooted daughters was inevitable, even though uptake of labelled nitrogen applied to daughters and its translocation towards mothers were measurable (evidencing uptake of nitrogen by very small roots of the daughters, Fig. 2).

At the time of the initial rapid daughter growth (2 weeks after the beginning of rooting), carbon translocation was directed towards daughters, with the shading treatment having no significant effect

(Fig. 3). The developing daughter roots and emerging daughter tillers thus probably formed the main sink for shared carbon reserves, and relative sink strength was not markedly affected by shading. Nitrogen translocation was balanced in both directions, with the shading treatment having no significant effect, although slower-growing shaded daughters seemingly still gained nitrogen support from mothers (Fig. 3). Total absolute nitrogen flow did not decline as we expected (Fig. 2). Daughter ramets therefore gained independence in terms of nitrogen uptake relatively soon after establishment, although the bidirectional flow between ramets had not ceased. In comparison, translocation of nitrogen was directed mainly towards the younger ramets in clonal fragments of *Fragaria chiloensis*, with only little reverse translocation (Alpert, 1996). However, bidirectional translocation, and thus net nutrient flow, has generally not been examined, which complicates comparisons of other studies with our results.

When daughters reached the size of mothers and ramet size was determined only by the shading treatment (8 weeks after rooting, Table 1), resource translocation patterns in *A. stolonifera* surprisingly seemed to be altered only by shading of mothers (Table 2). Carbon exchange between unshaded mothers and their developed daughters was balanced irrespective of daughter light conditions, while it was directed from shaded mothers towards daughters, which restricted export to light-limited mothers (Figs 2, 3). In contrast, net translocation of nitrogen was directed from developed daughters, whether shaded or not, towards mothers (Fig. 3). Although the effect of shading alone was not statistically significant for nitrogen translocation, the translocation towards unshaded mothers seems to be the main contributor to this directionality (Fig. 3). At the same time, light-limited mothers enhanced their proportional export of both resources towards daughters, and the reverse proportional export from daughters declined (Table 3). Similarly, the export of nitrogen towards unshaded ramets was enhanced by shading of labelled ramets in *Sasa palmata* (Saitoh *et al.*, 2006). Our results contrast with predicted higher translocation of photosynthates towards shaded ramets and only partly support the prediction of nitrogen translocation towards faster-growing, unshaded ramets at the late developmental stage (i.e. 8 weeks after rooting). On the other hand, translocation among established ramets has been shown to be enhanced by environmental resource gradients in several species (Marshall, 1990; Saitoh *et al.*, 2006), and the ability to support resource-limited ramets is considered one of the main advantages of clonal integration (Song *et al.*, 2013). For example, carbon translocation from mothers to 8-week-old daughters was enhanced by shading of daughters in *Alternanthera philoxeroides* (Xu *et al.*, 2010), and severe shading of mother ramets induced translocation of carbon from established daughters back to the mothers in *Lathyrus sylvestris* (Magda *et al*., 1988).

The translocation patterns that we observed indicate that the spread of clonal fragments of *A. stolonifera* to new sites is preferred to the maintenance of growth of developmentally older ramets in light-limiting conditions. On the other hand, when originally growing at a favourable patch, clonal fragments did not reallocate resources to already established daughter ramets and, instead, mother

ramets seemed to use nitrogen from newly occupied daughter patches to support their own growth. Younger ramets may therefore serve as a supplementary source of mineral nutrients for older ramets, as was also observed in *Populus tremuloides* (Pinno and Wilson, 2014), although the mother ramets were much larger than daughters in that case.

Contrary to our expectations, the magnitude of absolute carbon and nitrogen flows between ramets did not change significantly during daughter ramet development (although the proportions of exported labels declined, and net translocation markedly changed during development), and ramets thus remained physiologically interconnected, not only under heterogeneous conditions but also in homogeneous light conditions (Fig. 2). Consequently, translocation patterns in *A. stolonifera* are likely to respond readily to a change of local conditions or to stress. The maintained bidirectional nitrogen flow may partly be caused by different sites of energetically demanding reduction of nitrogen and its subsequent utilization (Li and Wang, 2011).

Our results also illustrate the necessity of bidirectional tracing of resource translocation to estimate net flows of resources in clonal plant systems. Although the translocation detected in one direction could be high, we have shown that it can be accompanied by an equally high reverse translocation, resulting in a near-zero net flow between ramets. To date, however, only a few studies have examined bidirectional translocation of labelled resources between ramets (Alpert *et al.*, 1991; Alpert, 1996; Pinno and Wilson, 2014).

Effect of integration on ramet growth

The positive effect of integration on the growth of daughter ramets (Fig. 4) probably reflects maternal support with both carbon and nitrogen at the initial stages of daughter development. The integration effect on daughter growth was not significantly modified by shading treatment, despite the observed effect of shading on the pattern of translocation. However, shading affected the translocation pattern only in the late stage of ramet development, which may have been too late to be detectable in terms of differences in biomass at the time of harvest. In mothers, there was a marginally significant indication of the cost of integration when daughters were shaded. However, this effect did not have a clear relationship with the observed translocation patterns of carbon and/or nitrogen between the ramets.

Similar to our results, shading of daughters affected neither the amount of translocated carbon nor the effect of integration on growth in *Phyla canescens* (Xu *et al.*, 2010). In contrast, both carbon import and the effect of integration on growth were higher in shaded daughter ramets of *Alternanthera philoxeroides* (Xu *et al.*, 2010, 2012). Only a few studies, however, have combined tracing of labelled elements with analyses of the effect of integration on growth (see D'Hertefeldt and Jonsdottir, 1994; Alpert, 1996; Saitoh *et al.*, 2006; Xu *et al.*, 2010), so it is not currently clear to what extent the resource translocation at different developmental stages is reflected in ramet growth.

Role of integration in competition for light

Enhanced benefits of integration under competitive conditions have been demonstrated for only a few plant species (Saitoh *et al.*, 2002; Roiloa *et al.*, 2010; Xu *et al.*, 2012; Wang *et al.*, 2016). In other studies, including ours, the overall benefits of integration on growth were not significantly altered by competition (e.g. Březina *et al.*, 2006; Yu *et al.*, 2009; Glover *et al.*, 2015), or there were even lower benefits of integration with versus without (above-ground) competition (Pennings and Callaway, 2000). We therefore suggest that in some species, integration may enhance performance of ramets under competition through their receipt of preferential support (Xu *et al.*, 2010). On the other hand, in other species, including *Agrostis stolonifera*, resources may be translocated among ramets to maximize efficient space exploration and exploitation of favourable patches. Nevertheless, it is not clear to what extent the resource-sharing strategy varies among species. It is conceivable that it may differ among species with different ecological strategies or among species from different environments, and therefore the current results should only be generalized with caution.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Variations in temperature and light intensity under full-light and shade treatments during the course of the experiment.

Fig. S2. Equipment used for labelling with $^{13}CO_2$.

Fig. S3. Example of a ramet pair before the final harvest.

Data deposition

Biomass and translocation data are available at Dryad Digital Repository.
https://doi.org/10.5061/dryad.f6q5j

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Paper III

Strategies of resource sharing in clonal plants: A conceptual model and an example of contrasting strategies in two closely related species

Jana Duchoslavová[1], Jan Jansa[2]

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[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 43 Praha 2, Czech Republic

[2] Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Praha 4, Czech Republic

Abstract

Background and Aims

Clonal growth helps plants to cope with environmental heterogeneity through resource integration via connecting organs. Such integration is considered to balance heterogeneity by translocation of resources from rich to poor patches. However, such an 'equalisation' strategy is only one of several possible strategies. Under certain conditions, a strategy emphasising acropetal movement and exploration of new areas or a strategy of accumulating resources in older ramets may be preferred. The optimal strategy may be determined by environmental conditions, such as resource availability and level of light competition. We aimed to summarise possible translocation strategies in a conceptual analysis and to examine translocation in two species from different habitats.

Methods

Resource translocation was compared between two closely related species from different habitats with contrasting productivity. The study examined the bidirectional translocation of carbon and nitrogen in pairs of mother and daughter ramets grown under light heterogeneity (one ramet shaded) at two developmental stages using stable-isotope labelling.

Key results

At the early developmental stage, both species translocated resources toward daughters and the translocation was modified by shading. Later, the species of low-productivity habitats, *Fragaria viridis*, translocated carbon to shaded ramets (both mother and daughter), according to the 'equalisation' strategy. In contrast, the species of high-productivity habitats, *Potentilla reptans*, did not support shaded mother ramets. Nitrogen translocation remained mainly acropetal in both species.

Conclusions

The two studied species exhibited different translocation strategies, which may be linked to the habitat conditions experienced by each species. The results indicate that we need to consider different possible strategies. We emphasise the importance of bidirectional tracing in translocation studies and the need for further studies to investigate the translocation patterns in species from contrasting habitats using a comparative approach.

Keywords:

Carbon, clonal plants, development, light, nitrogen, physiological integration, stable isotopes, translocation

Introduction

Plants cope with environmental heterogeneity, e.g. in resource availability, at a very local scale and have therefore evolved various traits, including specific morphological adaptations or phenotypic plasticity, to thrive under resource limitations. One of these strategies is clonal growth, which allows plants to spread horizontally via clonal organs such as stolons and rhizomes, explore new adjacent patches and integrate resources from a larger area through the interconnected plant parts (Marshall, 1990; Song *et al.*, 2013). Resource sharing among interconnected ramets (i.e. potentially independent plant parts rooting at different points) is considered one of the main advantages of clonal growth. Connection via clonal organs allow ramets to share water, mineral nutrients, and photosynthates and to transport signal molecules from ramet initialisation until the connection ends (Alpert *et al.*, 2002; Marshall, 1990; Pitelka and Ashmun, 1985). Initially, newly developing daughter ramets are supported by mother ramets because their resource demands cannot be covered by their limited resource uptake capacity. This support is analogous to maternal provision to seeds in sexual reproduction (Bullock *et al.*, 1994; Hartnett and Bazzaz, 1983; Wijesinghe, 1994). The initial maternal support, which appears to be universal across species, may change later in ramet ontogeny, with high intra- and interspecific variation (Alpert, 1999; Ma *et al.*, 2021; Xu *et al.*, 2010). Whereas persisting resource translocation among developed ramets may not be beneficial in stable, homogeneous habitats, it may be advantageous when resources are distributed heterogeneously in space or time (Alpert, 1999; Evans, 1991; Wang *et al.*, 2021). Different resource sharing strategies have been proposed depending on resource availability in the environment (Duchoslavová and Jansa, 2018; Kun and Oborny, 2003; Pitelka and Ashmun, 1985), but their systematic evaluation and direct experimental examination is missing.

Predicting translocation patterns under environmental heterogeneity is not straightforward, as different magnitudes and directions of resource translocation may be optimal in different contexts (Alpert, 1999; Pitelka and Ashmun, 1985). In this paper, we outline several possible distinct resource translocation strategies (known or expected) among developed ramets in heterogeneous environments, and we provide an example of different resource-sharing patterns in two closely related stoloniferous species. We propose names for the resource-sharing strategies, as we are not aware of any existing terminology that would be appropriate for this purpose, and we feel that the potentially contrasting patterns of resource translocation deserve to be named.

Hypothetical types of resource sharing

The hypothetical strategies differ in the pattern of resource sharing between established ramets that experience different resource availability, for example when plants grow laterally out of or into areas of denser vegetation or lower availability of belowground resources. In the first possible strategy, which we call the **Equalisation strategy**, developed ramets growing in resource-poor patches are

supported by ramets growing in resource-rich patches, regardless of their ontogenetic position (i.e. both acropetally and basipetally; Fig. 1a). It has been demonstrated in many studies (Alpert and Mooney, 1986; Evans, 1991; Shumway, 1995), and it has often been implicitly considered to be synonymous with clonal integration in the recent literature (Liu *et al.*, 2016; Wang *et al.*, 2021). Second, the **Acropetal translocation strategy** is characterized by mainly acropetal translocation of resources – developmentally older ramets are not supported even if they are resource-limited (Noble and Marshall, 1983; Xiao *et al.*, 2011; Fig. 1b). Support of younger ramets growing at lower resource level and no similar support of older ramets has been observed in several species (Portela *et al.*, 2021; Slade and Hutchings, 1987; van Kleunen and Stuefer, 1999; Xiao *et al.*, 2011). Third, daughter ramets could hypothetically be used as an extended hand of a mother ramet for a period of time, supporting the mother ramet's resource demands (the **Extended Hand strategy**; Guo *et al.*, 2020; Pinno and Wilson, 2014; Fig. 1c). Finally, plants might exhibit no net translocation between developed ramets (the **Zero net translocation strategy**). In this case, the clonal connection may be interrupted, or it may persist only as a remnant of the early translocation. Alternatively, the connection can be actively maintained in case of need, such as high stress or regeneration after disturbance (Schmid *et al.*, 1988; Wang *et al.*, 2017).

Which resource-sharing strategy is preferable may be determined by the nature of resource distribution and of plant competition in the environment (Alpert, 1999; Kun and Oborny, 2003; Pitelka and Ashmun, 1985). The Equalisation strategy may be beneficial to maintain long-lived ramets and compensate for temporary shortages, and may be particularly beneficial in the case of complementary heterogeneity of multiple resources (Alpert and Mooney, 1986; Friedman and Alpert, 1991; van Kleunen and Stuefer, 1999). With regard to light competition, the Equalisation strategy may be preferred when all individuals have a chance to reach the top of the canopy, as in the case of tall plants or in nutrient-poor habitats with low surrounding vegetation, since it enables the maintenance of established ramets (as suggested also by Wang *et al.*, 2021). The Acropetal translocation strategy, based on the horizontal acropetal movement of the plant body and exploration of new areas rather than prolonged support of resource-limited ramets, may be preferable especially when the environment is productive enough to allow rapid growth and patchy not only in space, but also in time (Gardner and Mangel, 1999; Pitelka and Ashmun, 1985), and when resources may get locally depleted. In habitats where the surrounding vegetation is typically taller than the focal plant and light competition is highly asymmetric, shaded ramets have a low chance of reaching the top of the canopy, and it may not be advantageous to invest resources in maintaining them. Light is thus scarce and patchily distributed in vegetation gaps for such shorter plants. The Acropetal translocation strategy could then allow rapid spread to new, potentially unshaded patches. Mainly acropetal direction of resource translocation may be driven by physiological constraints in some species (Stuefer, 1996), although the possibility of basipetal translocation has been demonstrated repeatedly (Jonsdottir and Watson, 1997; Shumway,

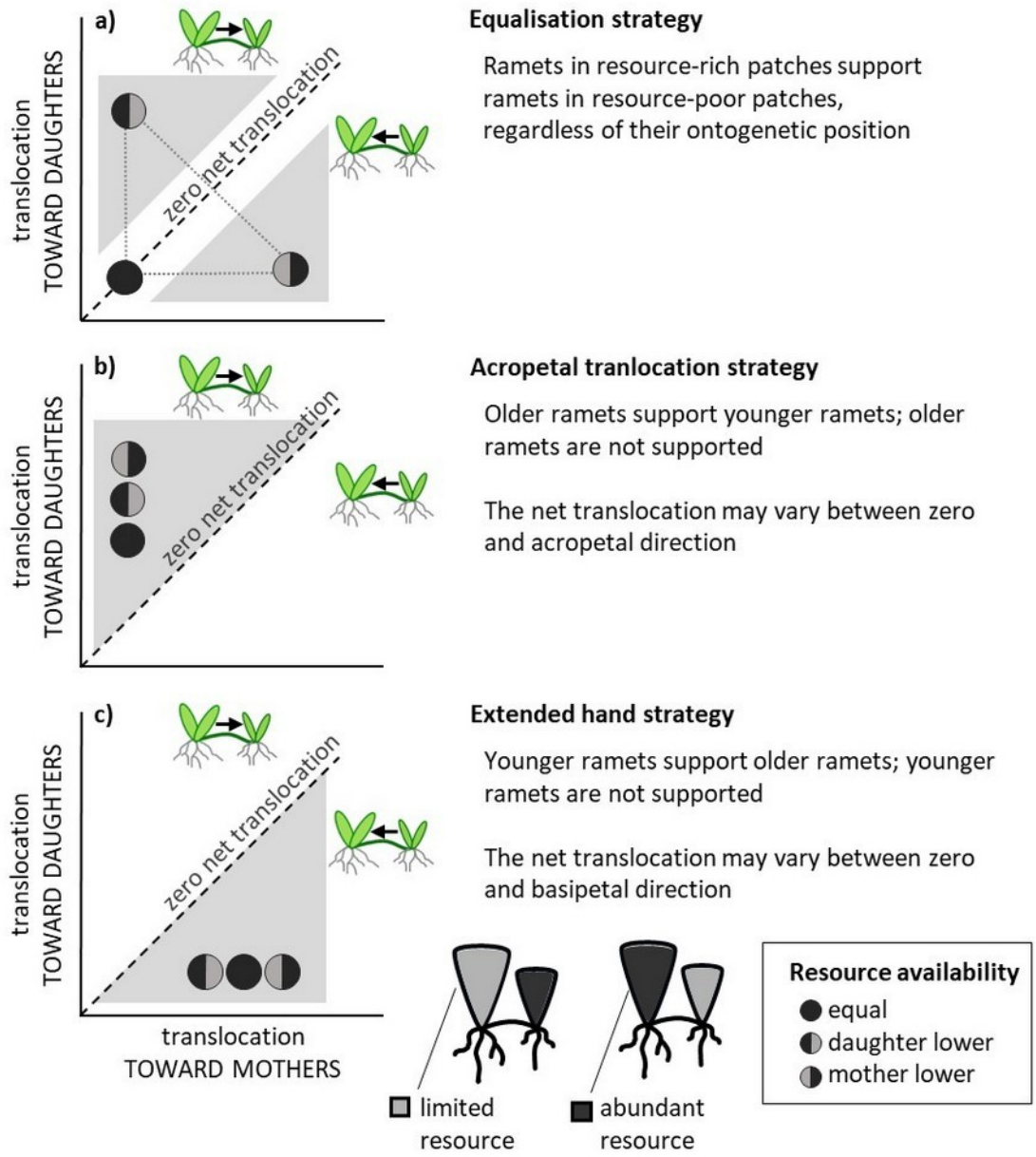

Figure 1 *Hypothetical* ***two-way translocation plots*** *for the proposed resource-sharing strategies between mother and daughter ramets in the later developmental stage. The dashed line indicates zero net translocation. Translocation toward daughters prevails in the zone above the dashed line, while translocation toward mothers prevails in the zone below the dashed line. Grey triangles indicate zones in which the values may range.*

1995; Tietema and van der Aa, 1981). Next, the Extended Hand strategy may be preferable when concentrating resources in the mother ramet brings benefits for the entire clonal fragment, such as when the mother ramet is flowering while the daughter ramets remain vegetative (Guo *et al.*, 2020). Presumably, this strategy may occur in species with long persisting ramets and young ramets remaining rather small and vegetative in the first year of their development. Due to internal sources of resource availability gradients, such translocation may be rather independent of environmental resource heterogeneity. We expect this strategy to be particularly effective for exploration of soil-borne

resources which might get depleted by older ramets. Finally, the Zero net translocation strategy is likely beneficial in homogeneous environments, or when cost of connection maintenance and benefits of clonal offspring fragmentation and dispersal outweigh the benefits of translocation.

While all the four translocation strategies have been observed, it is not yet clear how often they occur in clonal plants and how they depend on environmental conditions. Experiments evaluating the effects of clonal integration on ramet biomass provide a valuable indication of translocation patterns, but they usually do not separate the effects of translocation at early and late developmental stages (but see Ma *et al.*, 2021; Xu *et al.*, 2012). However, the translocation may be completely reversed during ontogeny, and the growth experiments thus do not prove the strategies directly. Tracing of labelled resources could provide direct evidence of the translocation strategy, but only if done under environmental heterogeneity and in both acropetal and basipetal direction. Nevertheless, this approach has been rarely used (but see (Duchoslavová and Jansa, 2018; Zhai *et al.*, 2022).

Experimental approach

To provide an example of resource-sharing strategies under light heterogeneity, we chose two closely related stoloniferous species from the Rosaceae family. We chose these species because they exhibit contrasting habitat preferences. *Fragaria viridis* inhabits dry grasslands with low vegetation, whereas *Potentilla reptans* can grow in more fertile habitats with higher vegetation, such as mesic meadows. Therefore, *F. viridis* is likely exposed to higher levels of light and lower availability of belowground resources in its natural habitats than *P. reptans* and may exhibit a different resource-sharing strategy.

In this study, we grew pairs of mother and daughter ramets of these species with one ramet unshaded and one ramet shaded by green shade simulating light competition. We traced labelled carbon and nitrogen in both directions to determine the resource-sharing strategies of the species. While carbon translocation is directly linked to light heterogeneity, nitrogen can be translocated to support photosynthesis in unshaded ramets as it constitutes an important part of chlorophyll (Saitoh *et al.*, 2006). Nitrogen tracing helped us gain a more complex picture of plants' resource economy, nutrient uptake capacity and relative resource limitation. In addition, we examined resource-sharing in early and later developmental stages of daughter ramets (2 weeks and 8 weeks after daughter-ramet initiation, respectively) to observe the change in resource-sharing patterns during ramet development.

We expected both species to translocate carbon and nitrogen toward daughter ramets in the early developmental stage. We hypothesized that unshaded daughters would form stronger sinks for nitrogen and therefore import more nitrogen than shaded daughters. In the later developmental stage, we expected carbon translocation to switch to the Equalisation strategy in *F. viridis* and to the Acropetal strategy in *P. reptans*. We hypothesized that nitrogen translocation would be directed toward unshaded mother or daughter ramets which are not limited by carbon, and likely form stronger sinks for nitrogen.

Materials and Methods

Species

Both experimental species belong to the Rosaceae family. They are perennial, form rosettes of leaves and spread horizontally via stolons with similar lateral spreading distances (20 cm for *F. viridis* and 18 cm for *P. reptans*; Klimešová *et al.*, 2017). Connections among ramets persist for the whole vegetative season. *Fragaria viridis* is common in pastures, dry grasslands, rocky steppes, and open forest edges (Slavík *et al.*, 1995; Ellenberg's indicator values of 7 for light and 4 for nitrogen; Chytrý *et al.*, 2018). *Potentilla reptans* occurs in mesic meadows, stream banks, ruderal areas, fields and forest edges (Slavík *et al.*, 1995; Ellenberg indicator values of 6 for light and 6 for nitrogen; Chytrý *et al.*, 2018). Therefore, *P. reptans* inhabits more nutrient-rich habitats than *F. viridis* and tolerates more shading.

Three genotypes per species were collected in the field from localities around Prague (Czech Republic) in 2016 and grown as source plants in an experimental garden at Charles University in Prague. Different genotypes were collected at locations at least 100 m apart from each other.

Initial cultivation

The stolons of source plants were placed on wet perlite to initiate rooting in late May 2017. After 2 weeks, individual ramets were separated and planted in 2 L pots with a mixture of washed sand and zeolite (1:1). The substrate was fertilised with slow-release fertiliser (Substral Osmocote for garden, 7 g per pot). These ramets are referred to below as the mother ramets. The pots were placed in a greenhouse equipped with supplemental lighting from 400 W metal halide lamps providing a minimum of 200 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR), extending the daylight period to 14 h. They were watered two times a day with tap water. Dead ramets were replaced by new ones until mid-June 2017. The plants were treated with insecticide to protect them against pests (specifically, *Tetranychus urticae*; the insecticides Nissorun and Karate were used as per the manufacturers' recommendations, www.agrobio.cz).

By early July, the mother ramets had produced one or more new stolons bearing several new rosettes of leaves. The initial size of the mother ramets was measured 1 day before the initialisation of daughter-ramet rooting as the number of leaves and number of stolons. The rooting of daughter ramets was initiated between 13–16 July 2017 by placing the longest stolon of each mother ramet in an adjacent vacant pot. The tips of these stolons were kept intact, and all the other stolons were left on plants, but they were not allowed to root (similarly to Alpert, 1999). All the unrooted stolons were kept in the same shade treatment as their associated ramet. Therefore, by a mother ramet we mean a developmentally older rooted rosette of leaves and all the unrooted stolons associated with this rosette, and by a daughter ramet we mean a developmentally younger rooted rosette of leaves and all the associated unrooted stolons. For logistic reasons, the initialisation of daughter-ramet rooting, as well

as labelling and harvesting, were conducted over 4 subsequent days. This made it possible to maintain the same intervals between the initialisation of daughter-ramet rooting and harvesting for all plants. Therefore, the ramet pairs processed at each harvest step (i.e. for a given developmental stage) were divided into 4 time-blocks with 1-d differences in rooting initialisation/harvest. The treatments were represented evenly among the blocks.

Experimental design

We used an experimental design with three shading treatments: (i) both ramets in full light, (ii) daughters shaded, and (iii) mothers shaded (Fig. 2). A shading cloth was installed 1 day before the initialisation of daughter-ramet rooting. We used the shading cloth combined with 3-cm-wide strips of green foil (LeeFilters Fern Green 122, www.leeflters.com) to shade the plants from the top and all sides, thus simulating the changes in both light quantity and quality caused by aboveground competition (80% PAR reduction and 30% reduction of the red to far-red ratio, as measured by the spectroradiometer Avaspec-2048, VA300; see Fig. S1 for a picture of the shading setup; Duchoslavová & Jansa, 2018).

The bidirectional translocation of carbon and nitrogen between mothers and daughters was examined by stable-isotope pulse labelling (using ^{13}C and ^{15}N) at two developmental stages of the daughters: 2 weeks after the initialisation of rooting (early developmental stage) and 8 weeks after the initialisation of rooting (later developmental stage). In each ramet pair, either translocation to daughter ramets or translocation to mother ramets was measured. Four replicates were used for each combination of species, shading treatment, measured direction of translocation and time of labelling.

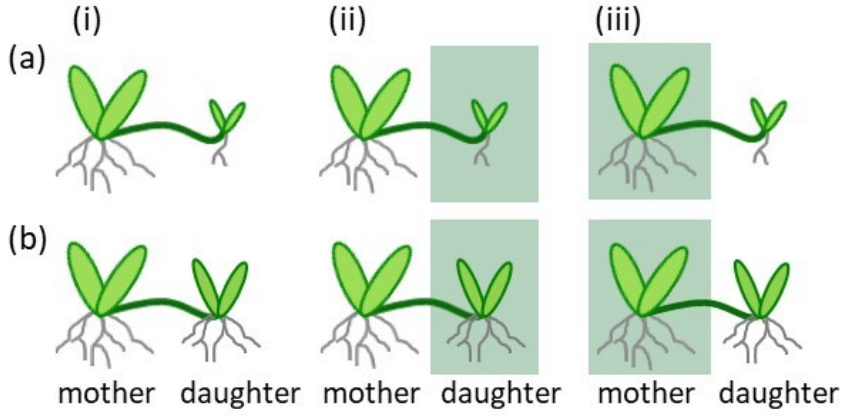

Figure 2 *Three shading treatments with green shade were used in the experiment: full light (i), daughter shaded (ii) and mother shaded (iii). The plants were pulse-labelled by stable isotopes of C and N and harvested in two developmental stages of daughter ramets: (a) the early developmental stage (2 weeks after initialisation of daughter rooting) and (b) the later developmental stage (8 weeks after initialisation of daughter rooting). Note that new unrooted stolons forming on both mother and daughter ramets are not depicted in this scheme.*

Stable-isotope labelling

Plants were pulse-labelled simultaneously by nitrogen (^{15}N) and carbon (^{13}C) according to a protocol used in a previous experiment (Duchoslavová and Jansa, 2018). Labelling began at approximately 8:00 a.m. Nitrogen was applied directly to the substrate of a labelled ramet by a syringe in the form of doubly labelled ammonium nitrate (99 atom% ^{15}N, 15 mg per pot). Carbon was applied in the form of $^{13}CO_2$. Pots with labelled ramets were enclosed in plexiglass chambers equipped with a fan to mix the inner atmosphere, and 20 ml of phosphoric acid (20%, w:v) was injected into a vial with ^{13}C-enriched sodium carbonate (99% atom% ^{13}C, 0.3 g per pot) to release $^{13}CO_2$. The calculated initial $^{13}CO_2$ concentration inside the chambers reached 3,100 ppm. The ramets were allowed to assimilate labelled carbon for 2 hours, and the remaining CO_2 in the atmosphere was then scrubbed by circulating the air through an NaOH solution (0.1 M, 200 ml). The labelling took place in full sunlight supplemented by additional light from diode lamps (LumiGrow Pro Series Pro 325 LED Lighting Systems, photosynthetic photon flux density 333 μmol m^{-2} s^{-1} at a distance of 1 m), and the plants were returned to experimental shading conditions immediately after the labelling period.

Labelled ramet pairs were harvested exactly 2 days after labelling. This period enables labelled elements to go through the entire day cycle but still reflects actual (short-term) rather than cumulative (long-term) resource translocation. Mother and daughter ramets were separated, and the roots were washed and separated from the shoots. Unrooted stolons were kept on the plants and considered as part of the shoots. The plant material was then dried (65 °C for 2–3 days), weighed and ground to a fine powder using a ball mill (MM200, Retsch, Haan, Germany) before the elemental and isotopic analyses. The N and C concentrations and isotopic composition of the two elements were measured using an elemental analyser (Flash EA 2000) coupled with an isotope ratio mass spectrometer (Delta V Advantage, ThermoFisher Scientific, Waltham, MA, USA).

To calculate the amount of ^{13}C and ^{15}N originating from labelling pulse (i.e. excess ^{13}C or ^{15}N), F-ratios, i.e. the proportion of the heavy isotope in the total amount of the element, were calculated as $R_S/(R_S+1)$, where R_S is the molar isotope ratio in a sample ($^{15}N/^{14}N$ or $^{13}C/^{12}C$). The amount of total carbon or nitrogen a sample (C, in moles) was then calculated as follows:

$$(1)\ C = \frac{DW \times B}{a \times F + b \times (F-1)},$$

where DW is the dry weight of the sample, B is the molar concentration of carbon or nitrogen in a sample, a is 12 for carbon and 14 for nitrogen, b is 13 for carbon and 15 for nitrogen, and F stands for the F-ratio of the respective element in a sample, as specified above. F-ratios of ^{13}C or ^{15}N originating from pulse-labelling (F_D) were calculated as

$$(2)\ F_D = F_S - F_L,$$

where F_S is a sample's F-ratio and F_L is a limit F-ratio below which we cannot detect the stable-isotope enrichment with a sufficient confidence. This value was calculated from the F-ratios of the unlabelled control samples as 99th percentile of an approximated normal distribution. Therefore, F-ratio values above this limit would come from this distribution with probability less than 1 %. Negative F_D values were replaced by zeroes. This approach resulted in less statistically significant but more credible results than F_D calculated as the difference between the sample F-ratio and the mean F-ratio of unlabelled controls.

The amount of ^{13}C and ^{15}N originating from pulse-labelling (E, in moles) was finally calculated as

$$(3) \quad E = F_D \times C.$$

The amount of ^{13}C and ^{15}N originating from pulse-labelling in unlabelled ramets (roots and shoots combined) is further referred to as the amount of translocated ^{13}C and ^{15}N.

As all ramets were labelled in full-light conditions to allow for sufficient incorporation of ^{13}C into plant biomass, excess ^{13}C values of shaded ramets and corresponding amounts of translocated ^{13}C might be overestimated. However, the qualitative pattern of ^{13}C translocation over the subsequent two-day period and fractions of ^{13}C exported to the other ramet were presumably unaffected by the labelling conditions.

Data analyses

We performed statistical analyses in the R statistical environment using linear mixed-effects models (lmerTest package; Kuznetsova *et al.*, 2017). These models enabled the description of the data's hierarchical structure, which was caused by the use of several genotypes. P-values were provided via Satterthwaite's degrees of freedom method. For the analysis of ramet biomass, the effects of initial number of leaves, initial number of stolons, ramet (mother/ daughter), shading, species, and their interactions were modelled in a separate model for each developmental stage. Genotype identity was included as a random effect affecting the intercept. The biomass was log-transformed in order to meet the model assumptions. For the analyses of translocation, the effects of species, traced translocation direction, shading and their interactions on translocated ^{13}C and ^{15}N for the two developmental stages were modelled in four separate models (one model for each combination of resource and developmental stage). Genotype identity was included as a random effect affecting the intercept, and its effect was allowed to change with direction and shading. The translocated ^{13}C and ^{15}N were log-transformed in all models in order to meet the model assumptions.

Results

Ramet size and uptake of carbon and nitrogen

At the early developmental stage (i.e. 2 weeks after daughter rooting initialisation), there were no pronounced differences in ramet biomass between the two species. Mothers in the full-light treatment reached 4.6 times higher average dry biomass than daughters. Shading the daughters reduced biomass of both ramets by 40%. Shading the mothers reduced biomass of mothers by 50% in *F. viridis* and by 30% in *P. reptans* while it reduced biomass of daughters only in *F. viridis* by 40% (Fig. 3, Table S1). In terms of carbon uptake, mothers in the full-light treatment assimilated on average 3.1 times more labelled carbon per ramet than daughters in full light. Shading had a similar effect on carbon uptake as it did on biomass (Fig. S2). It should be noted that the carbon uptake values represent the potential uptake capacity of the ramets measured in full light for all ramets. Nitrogen uptake was very low in young daughters, leading to disproportionally higher uptake by mothers (7 and 23.6 times in *F. viridis* and *P. reptans*, respectively). This was likely caused by the markedly lower root mass fraction of daughters in comparison to mothers (Fig. S3). On average, *P. reptans* mothers assimilated 2 times more labelled nitrogen than mothers of *F. viridis* at the time of the first harvest (Fig. S2).

At the later developmental stage (i.e. 8 weeks after daughter rooting initialisation), mothers of *F. viridis* and *P. reptans* in the full-light treatment had 1.9 and 1.5 times higher average dry biomass than daughters, respectively. Shading reduced the biomass of the shaded ramets by 60% times in both ramets of *F. viridis* and by 30% in both ramets of *P. reptans.* Moreover, shading the mothers reduced daughter biomass by 50% in *F. viridis* and by 20% in *P. reptans* (Fig. 3, Table S1). Shading the daughters did not reduce the biomass of mothers in either species (Fig. 3). Despite the differences in biomass, daughter and mother ramets assimilated on average similar amount of labelled carbon in both species. Shading treatments affected the carbon uptake capacity only to a limited extent (Fig. S2). Nitrogen uptake was proportional to root mass of labelled ramets ($R^2 = 0.83$, data not shown) and remained higher in mother ramets at the later developmental stage. In the full-light treatment, mothers assimilated on average 1.5 times more labelled nitrogen than daughters in both species, with *P. reptans* assimilating 1.3 times more than *F. viridis*. At the later developmental stage, the effect of shading on nitrogen uptake was similar to its effect on biomass (Figs 3 and S2).

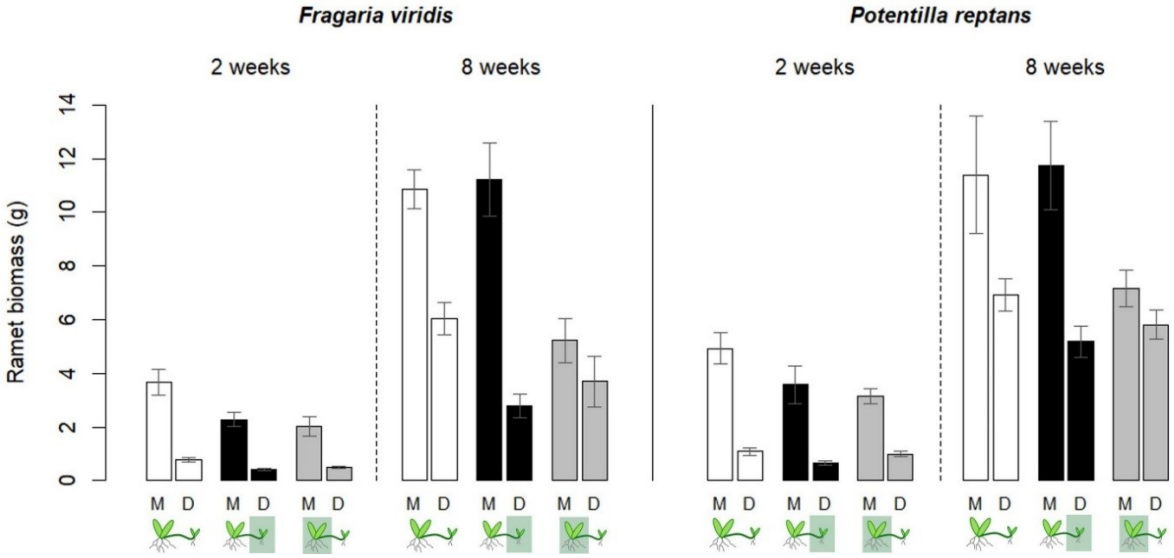

Figure 3 *Biomass of mother (M) and daughter (D) ramets in early and later developmental stages of daughter ramets (i.e. 2 and 8 weeks after daughter initialisation). Means and SEM are depicted.*

Carbon translocation

At the early developmental stage, carbon was translocated significantly more from mothers to daughters than from daughters to mothers. This effect was significantly modified by shading treatment. Specifically, shading the mothers increased translocation directionality toward mothers in both species (Table 1, Fig. 4).

At the later developmental stage, the net flow of carbon in the full-light treatment was near zero in both species. However, the two species significantly differed in their response to shading treatment. While shading of daughters seemed to promote translocation toward daughters in both species, they responded differently to shading of mothers. In *F. viridis*, shading of mothers increased carbon translocation from daughters to mothers. In contrast, in *P. reptans*, shading of mothers reduced carbon translocation from daughters to mothers to undetectable values, while it did not markedly alter translocation to daughters (Table 1, Fig. 4, see Fig. S4 for individual values).

In relative terms, mother ramets of *F. viridis* and *P. reptans* in the full-light treatment exported on average 5.7 and 2.6% of assimilated carbon, respectively, to young daughters at the early developmental stage. At the later stage, the exported fractions were generally lower. Mother ramets of *F. viridis* and *P. reptans* exported 2.4% and 1.5% of assimilated carbon to shaded daughters, respectively. Daughters of *F. viridis* and *P. reptans* exported 4.7% and 0% of assimilated carbon to shaded mothers, respectively (Fig. 4). For detailed information on the exported proportions, see Table S2 in Supplementary Information.

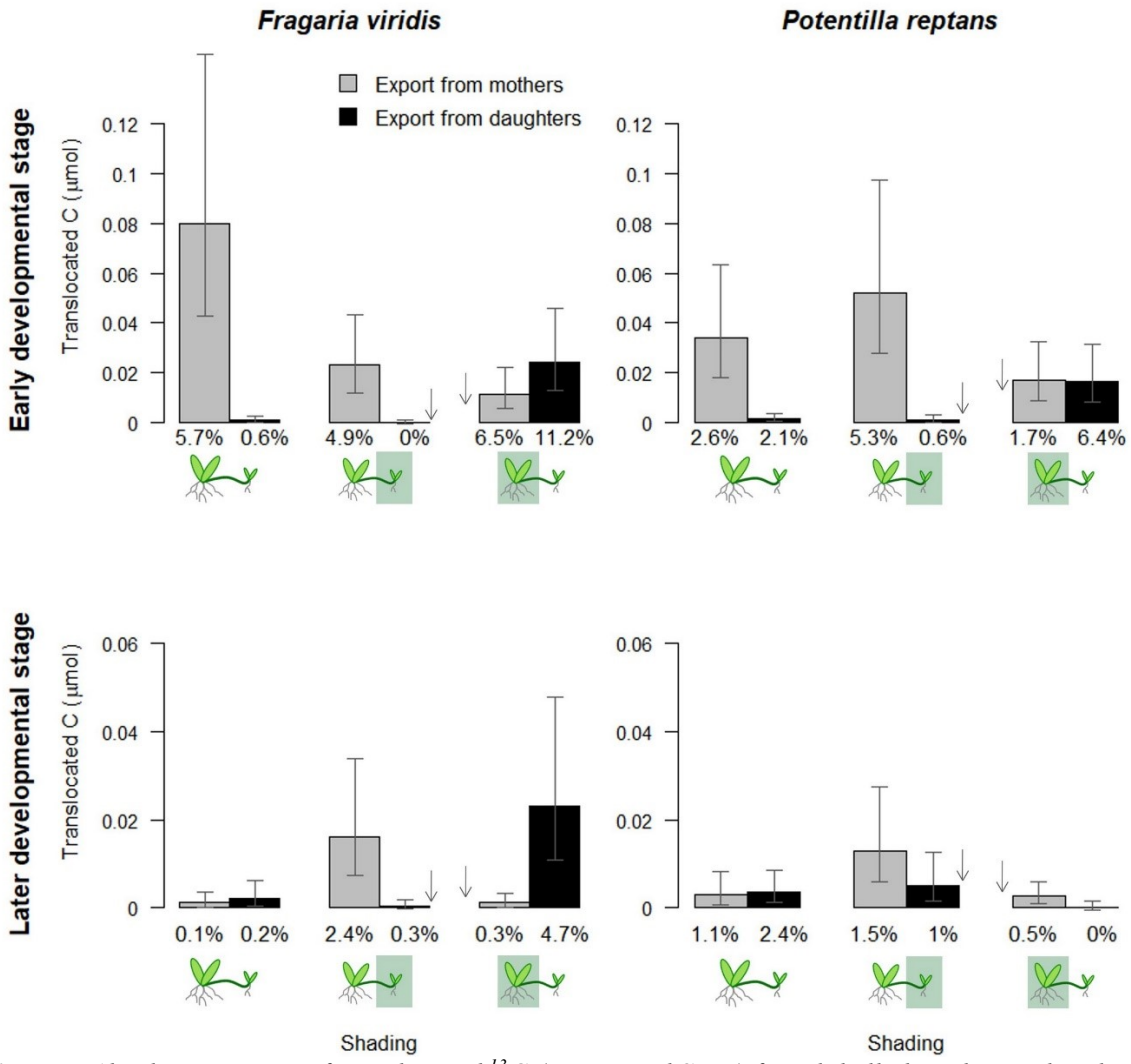

Figure 4 *Absolute amounts of translocated ^{13}C (means and SEM) from labelled mother to daughter ramets and from labelled daughter to mother ramets. Numbers under bars indicate mean fractions of ^{13}C exported from labelled ramets toward unlabelled ramets. Arrows next to the bars indicate which absolute values have possibly been overestimated by the labelling method and the direction where more realistic values may lie. Note that y-axis range for the later developmental stage is half of that for the early developmental stage. Values of absolute amounts are based on linear models with logarithmic transformation (values were back-transformed).*

Nitrogen translocation

At the early developmental stage, nitrogen was only translocated toward daughters in both species, translocation from daughters to mothers was not detectable. *P. reptans* mothers translocated significantly more nitrogen to daughters than *F. viridis* mothers, in proportion to their higher nitrogen uptake. For both species, translocation to daughters was highest in the full-light treatment and lowest when the daughters were shaded (Table 1, Fig. 5).

At the later developmental stage, nitrogen translocation toward daughters was still significantly higher in both species. Translocation to mothers was detectable, at least in some plants, and *P. reptans* tended to have higher translocation to mothers. However, differences between the species and shading treatments were not significant due to high variation (Table 1, Fig. 5).

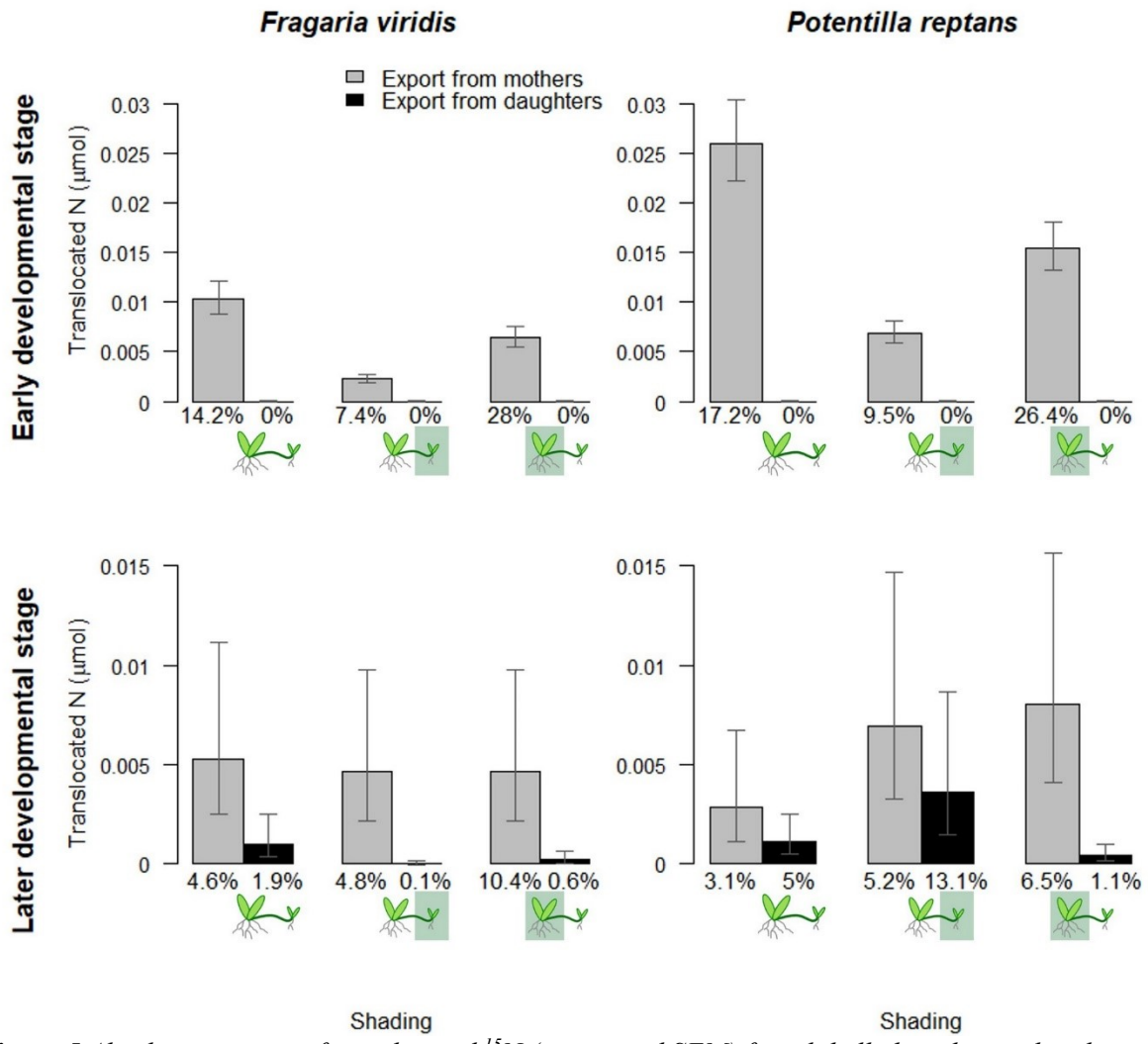

Figure 5 *Absolute amounts of translocated ^{15}N (means and SEM) from labelled mother to daughter ramets and from labelled daughter to mother ramets. Numbers under the bars indicate mean fractions of ^{15}N exported from labelled ramets toward unlabelled ramets. Note that y-axis range for the later developmental stage is half of that for the early developmental stage. Values of absolute amounts are based on linear models with logarithmic transformation (values were back-transformed).*

The mean proportions of labelled nitrogen exported from mother ramets to early-stage daughters under homogeneous conditions were 14.2% for *F. viridis* and 17.2% for *P. reptans*. These proportions were modified by shading; with the highest proportions exported from shaded mothers and the lowest from unshaded mothers to shaded daughters. In the later stage, the mean exported proportions of nitrogen were 4.6 and 3.1% for mother ramets of *F. viridis* and *P. reptans,* respectively, under homogeneous conditions. These proportions increased to 10.4 and 6.5% when the mothers were shaded. The mean proportions exported by daughters ranged from 0.1% to 13.1% of labelled nitrogen, the latter exported by shaded *P. reptans* daughters (Fig. 5). See Table S2 in Supplementary Information for further details.

Table 1 *ANOVA table of the linear mixed-effects models of C and N translocation. Note the corrected values of denominator degrees of freedom (see Methods for details).*

Translocated ^{13}C

	First harvest					
	d.f.	SumSq	MeanSq	DenDF	Fvalue	Pr(>F)
Species	1	0.72	0.72	10.46	0.54	0.478
Direction of translocation	**1**	**48.03**	**48.03**	**31.57**	**35.94**	**<0.001**
Shading	2	4.09	2.05	5.28	1.53	0.299
Species x direction	1	0.03	0.03	31.57	0.02	0.877
Species x shading	2	2.00	1.00	5.28	0.75	0.517
Direction x shading	**2**	**32.86**	**16.43**	**31.57**	**12.30**	**<0.001**
Species x direction x shading	2	1.75	0.88	31.57	0.66	0.526
	Second harvest					
	d.f.	SumSq	MeanSq	DenDF	Fvalue	Pr(>F)
Species	1	0.03	0.03	3.49	0.02	0.907
Direction of translocation	1	0.87	0.87	29.93	0.51	0.483
Shading	2	3.00	1.50	8.93	0.87	0.452
Species x direction	1	1.78	1.78	29.93	1.03	0.318
Species x shading	2	7.34	3.67	8.93	2.12	0.176
Direction x shading	**2**	**11.56**	**5.78**	**29.86**	**3.35**	**0.049**
Species x direction x shading	**2**	**12.95**	**6.47**	**29.86**	**3.75**	**0.035**

Translocated ^{15}N

	First harvest					
	d.f.	SumSq	MeanSq	DenDF	Fvalue	Pr(>F)
Species	**1**	**0.86**	**0.86**	**4.57**	**18.22**	**0.010**
Direction of translocation	**1**	**56.56**	**56.56**	**4.38**	**1200.73**	**<0.001**
Shading	**2**	**1.01**	**0.51**	**3.85**	**10.71**	**0.027**
Species x direction	**1**	**0.63**	**0.63**	**4.38**	**13.26**	**0.019**
Species x shading	2	0.01	0.01	3.85	0.10	0.903
Direction x shading	**2**	**3.85**	**1.93**	**21.51**	**40.88**	**<0.001**
Species x direction x shading	2	0.02	0.01	21.51	0.24	0.787
	Second harvest					
	d.f.	SumSq	MeanSq	DenDF	Fvalue	Pr(>F)
Species	1	3.80	3.80	5.26	2.07	0.207
Direction of translocation	**1**	**42.14**	**42.14**	**30.25**	**22.90**	**<0.001**
Shading	2	0.20	0.10	5.70	0.05	0.948
Species x direction	1	4.06	4.06	30.25	2.20	0.148
Species x shading	2	7.41	3.71	5.70	2.01	0.218
Direction x shading	2	4.25	2.13	29.86	1.16	0.329
Species x direction x shading	2	4.99	2.49	29.86	1.35	0.273

Discussion

Using a pulse-chase labelling experiment, we showed transition from an early to a later pattern of resource sharing between ramets and identified two different later-stage patterns of carbon translocation in the two relative clonal species growing in habitats with different ecological regimes. Specifically, in *F. viridis,* carbon was translocated to shaded ramets at the later developmental stage, consistently with the Equalisation strategy. In contrast, *P. reptans* daughters did not export any carbon to shaded mothers, consistently with the Acropetal translocation strategy. Nitrogen translocation was mainly acropetal and its pattern did not differ significantly between *F. viridis* and *P. reptans*, as discussed below.

Early developmental stage of ramets

Physiological integration between mother and daughter ramets is inevitable at the early developmental stage, especially for soil-borne resources, as daughters' resource-acquiring organs are still developing (e.g., Duchoslavová and Jansa, 2018; Hartnett and Bazzaz, 1983; Ma *et al.*, 2021). In our experiment, shading of one ramet had very similar effect on biomass of both ramets suggesting high interdependence of ramets at the early developmental stage. Daughter ramets of both species studied were markedly smaller than mothers, had a lower uptake capacity for carbon and a very low (but detectable) uptake capacity for nitrogen. Accordingly, carbon and nitrogen translocation was predominantly directed to daughters in both species. However, even at the early developmental stage, shaded mothers did not appear to be a relatively stronger carbon source than unshaded daughters, as zero net carbon translocation was observed in this treatment. The acropetal nitrogen translocation followed the expected source-sink relations, with the lowest absolute translocation toward slower growing shaded daughters. Highest fractions of assimilated nitrogen were exported from shaded mothers to unshaded daughters, presumably to support their photosynthetic capacity (Saitoh *et al.*, 2006), although the absolute amounts did not exceed translocation in the homogeneous treatment. In summary, shading conditions had significant effects on the translocation of both carbon and nitrogen during early development, which has rarely been investigated (Duchoslavová and Jansa, 2018).

Later developmental stage of ramets

At the later developmental stage of ramets, daughter ramets were still significantly smaller than mothers, but generally had comparable carbon uptake capacity. Our results showed zero net translocation of carbon in homogeneous conditions, as did the previous study on *A. stolonifera* (Duchoslavová & Jansa, 2018). Under heterogeneous conditions, persistent directed translocation of carbon occurred, consistent with previous findings (Saitoh *et al.*, 2002; Wang *et al.*, 2021). Our results further showed that bidirectional resource equalisation is only one of multiple possible translocation strategies in heterogeneous conditions. *F. viridis* translocated carbon toward shaded ramets regardless of their developmental position, in accordance with the Equalisation strategy. In contrast, carbon

translocation toward shaded mother ramets stopped completely in *P. reptans* or *A. stolonifera* (Duchoslavová and Jansa, 2018). When only daughters were shaded, the net translocation flow was slightly directed toward daughters in these species (Fig. 6). Thus, the latter two species did not support developmentally older ramets and they seem to support developmentally younger ramets growing in less favourable conditions less extensively than *F. viridis*. This pattern is in accordance with the proposed Acropetal strategy of late resource sharing. The lack of support of older, resource-limited ramets in some species has previously been suggested by growth experiments (e.g., Xiao *et al.*, 2011), but not demonstrated by labelling studies.

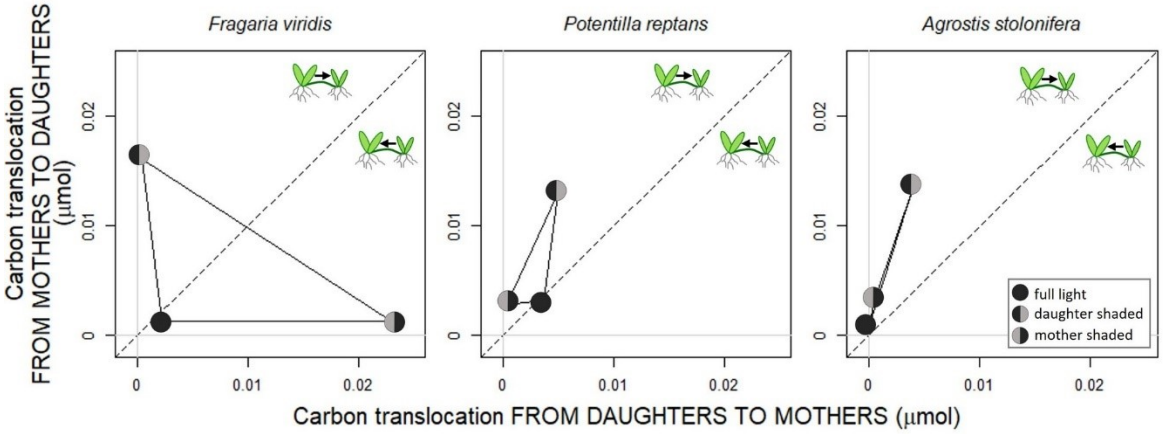

Figure 6 *Two-way carbon translocation plots for the three examined species at the later developmental stage of daughter ramets (data for* A. stolonifera *from Duchoslavová and Jansa, 2018). Means are depicted, see Fig. 4 for SEM.*

Regarding carbon source-sink relationships, larger and unshaded ramets presumably formed stronger sources whereas growing tissues formed main sinks for carbon (and nitrogen). Both the mother and daughter ramets were composed of rooted rosettes of leaves and unrooted stolons with leaves. On the one hand, the unrooted stolons may have acted as strong sinks driving translocation (Alpert, 1999; Ginzo and Lovell, 1973; Golovko *et al.*, 2004), but on the other hand they may have been photosynthetically self-sufficient due to their developed leaves. Several cases of unrooted and poorly rooted daughters tended to show higher carbon translocation to mothers (Fig. S4), suggesting an independence of unrooted stolons in carbon uptake, consistent with our previous findings in *Agrostis stolonifera* (Duchoslavová and Jansa, 2018).

Differences in nitrogen uptake between mothers and daughters at the later developmental stage remained more pronounced than in the case of carbon uptake, especially when daughters were shaded, and were driven by root mass of labelled ramets. Shading thus reduced nitrogen availability for ramets via its effect on growth (see also Freschet *et al.*, 2018), although nitrogen levels were not manipulated in the experiment. The lower uptake of nitrogen in daughters, together with strong nitrogen sinks in the growing tissues of daughters, likely led to prevailing nitrogen translocation toward daughters in both species. Nitrogen translocation showed high variability, did not significantly differ between the two species and, in contrast to the early developmental stage, was not significantly affected by shading

at the later developmental stage. This result did not confirm our expectations nor an observed effect of light heterogeneity on nitrogen translocation in *Sasa palmata*, where enhanced nitrogen translocation toward ramets in open patches likely increased the photosynthetic activity of illuminated leaves (Saitoh *et al.*, 2006).

Ecological consequences of the observed translocation strategies

The late-stage carbon sharing strategies showed in this study are manifested under horizontal light heterogeneity, which may occur in patchy and sparse vegetation or in gaps in vegetation created by local disturbances. Support of younger shaded ramets, which was observed in both species, may increase the competitive ability of clonal plants growing from an open area into the shaded conditions of a plant community, as was demonstrated in an experiment with *Fragaria chiloensis* (Wang *et al.*, 2021). On the other hand, clonal growth is a way to colonise gaps in vegetation (Kohler *et al.*, 2006; Macek and Lepš, 2003; Vítová *et al.*, 2017), and the Acropetal translocation strategy may be associated with gap exploration and colonisation, especially when belowground resources are not limiting. Accordingly, *P. reptans* has been shown to adjust its growth response to the height and density of simulated neighbours, preferring lateral spread in the presence of tall neighbours (Gruntman *et al.*, 2017).

Although the observed translocation strategies confirmed our expectations, the proposed connection between translocation strategy and habitat conditions, which implies different selection pressures, needs further testing by a comparative study. Although closely related, the two species also differ in other aspects, such as the positioning of flowers on stolons (*P. reptans*) or on rooted rosettes of leaves (*F. viridis*). This could lead to an alternative explanation of the different carbon translocation patterns at the later developmental stage, with *F. viridis* supporting older shaded ramet to promote their sexual reproduction (Alpert *et al.*, 2002).

Effect of translocation patterns on growth

Most clonal translocation studies have measured the growth characteristics of plants instead of using labelled-element tracing, which is more technically and financially demanding. The link between resource-sharing pattern and growth response is crucial for understanding the impacts of translocation strategies. Unfortunately, only a few studies have provided both tracing and growth data for the same experimental setup (see D'Hertefeldt and Jonsdottir, 1994; Duchoslavová and Jansa, 2018; Xu *et al.*, 2012, 2010). Xu and collaborators (2010) obtained matching results in two creeping species for the integration effect on daughter-ramet growth and late-stage carbon translocation toward daughters. However, determining the translocation strategy from growth data is complicated in later developmental stages by initial acropetal translocation, which may overlap the effect of the later translocation pattern (Duchoslavová and Jansa, 2018).

The proportions of recently assimilated resources that are exported may provide an alternative way of estimating possible translocation impacts and help interpret absolute amounts of translocated resources. Pitelka and Ashmun (1985) considered an export of 1–5% of a resource to have a significant effect on growth, reproduction, or survival. In conditions distinguishing the two strategies in our experiment (i.e. in the later developmental stage under heterogeneous light), *Fragaria* translocated on average 2.4 and 4.7% of assimilated carbon to shaded daughter and mother ramets, respectively. This was comparable to its initial maternal export constituting 4.9 to 6.5% of assimilated carbon. We thus consider this later translocation in *Fragaria* to have a significant effect on plant functioning. However, later-stage translocation flows in *Potentilla* were less clear – carbon translocation between mothers and shaded daughters occurred in both directions and only 0.5% of assimilated carbon was translocated from shaded mothers. Effect of such translocation rates on growth of established daughters may be questionable and, therefore, the most distinctive characteristic of *Potentilla* resource-sharing pattern is the absence of translocation toward shaded developmentally older ramets (Fig. 4).

Methodological issues

Despite the number of translocation studies, it is difficult to identify translocation strategies in the literature. We see two major reasons for this. First, translocation in the Equalisation or Acropetal translocation strategy does not differ in homogeneous conditions. Therefore, it is not possible to distinguish these two translocation strategies in studies that do not involve environmental gradients. Nevertheless, many tracing studies use homogeneous conditions that do not allow the identification of resource-sharing strategies (e.g., Alpert, 1996; D'Hertefeldt and Jonsdottir, 1999; Ginzo and Lovell, 1973). Further, our results demonstrate the necessity of bidirectional tracing for the recognition of translocation strategies. Basipetal translocation of carbon showed higher plasticity than acropetal translocation in the studied species; acropetal carbon translocation alone (Fig. 4, grey bars) would not reveal marked differences. However, many studies have traced labelled resources in only one direction, and important parts of the story may thus remain hidden (Saitoh *et al.*, 2006; Xu *et al.*, 2010). Similarly, the necessity of bidirectional tracing for estimating the carbon budget was recognised in a system of plant and mycorrhizal fungi (Cameron *et al.*, 2008).

Bidirectional tracing of carbon translocation between ramets in different light regimes, however, poses some methodological challenges. We decided to label the plants in full-light conditions to be able to detect the translocation flow, although the uptake of carbon by shaded ramets was thus possibly overestimated to some extent. The possible overestimation is reflected in the result interpretation, and it could not have affected observed differences between the species.

Although we traced the translocation of both carbon and nitrogen in our experiment, we only manipulated the availability of light. It is likely that translocation strategies under environmental

heterogeneity in soil-borne nutrients also vary between species and may be influenced by the productivity of the species' habitats. However, competition for nutrients does not switch from symmetric to asymmetric, as in the case of light competition. Translocation strategies under nutrient heterogeneity may differ from those found under light heterogeneity due to the different nature of competition for these resources.

Conclusion

The two different resource-sharing strategies observed demonstrated that the pattern of resource translocation in clonal plants is not a simple function of resource availability. Comparative studies involving multiple species are necessary to test the relationship between species' resource-sharing strategy and typical environment. We see the experiment presented here as a first step towards this goal. It is important to note that accurate comparisons can only be made through bidirectional translocation measurements, which involves measuring translocation from both mother to daughter and daughter to mother.

Supplementary data

The following supplementary data are available online.

Figure S1. Photograph of experimental setup.

Table S1. ANOVA table of linear mixed-effects models of ramet biomass.

Figure S2. Total assimilated ^{13}C and ^{15}N.

Figure S3. Root mass fraction of ramets at the early and later developmental stages.

Figure S4. Individual values of translocated ^{13}C.

Table S2. Fractions of ^{13}C and ^{15}N exported from labelled ramets toward unlabelled ramets.

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Conflict of interest

No conflict of interest declared.

Author contributions

Jana Duchoslavová planned the experimental design, conducted the experiment, analysed the data and wrote the manuscript. Jan Jansa advised on the experimental design and provided the elemental and

isotopic analyses. Both authors contributed critically to the drafts and gave final approval for publication.

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Paper IV

Nitrogen sharing strategies in six clonal species

Jana Duchoslavová[1]

[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 43 Praha 2, Czech Republic


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Abstract

Nitrogen is often a limiting factor for plant growth, and its availability is a major determinant of level of competition. In clonal plants, patterns of nitrogen translocation between ramets may be part of plant nitrogen economics, and, as such, may also be related to the typical availability of nitrogen. In nutrient-poor habitats, extensive nutrient sharing balancing resource availability may be important, whereas nutrient sharing between established ramets may not be beneficial in productive habitats.

I tested the proposed nutrient sharing strategies on nitrogen translocation in six stoloniferous species that occur in habitats of varying productivity. Mother and daughter ramets of each species were grown either in a homogeneous nutrient-poor treatment or in a "nutrient-poor to nutrient-rich" treatment. I traced the translocation of nitrogen in both directions using stable isotope labelling when the daughter ramets were one month old.

Surprisingly, I found no effect of nutrient treatment on nitrogen translocation. Instead, each species translocated nitrogen either acropetally, basipetally, or equally in both directions. There was no relationship between the direction of translocation and the productivity of the species' habitats. However, net translocation seemed to be related to the relative size of daughters across species, and within *Veronica officinalis*.

The results suggest that the relative size of plant parts is an important determinant of the strength of the sink for nitrogen they form, and that the growth habit of a species can affect its nitrogen translocation. Under certain conditions, such internally induced source-sink relationships may dominate over external nitrogen heterogeneity. I speculate that growth habit, together with nitrogen translocation patterns, may be part of adaptive growth strategies.

Key words: clonal plants, clonal integration, stolons, nitrogen, pulse labelling, stable isotopes, environmental heterogeneity, productivity, translocation

Introduction

As autotrophic organisms, plants obtain both carbon and mineral nutrients for their growth directly from the abiotic environment. Mineral nutrients are taken up from the soil by plant roots and distributed within the plant to sites of use or storage (Tegeder and Masclaux-Daubresse, 2018). The distribution of mineral nutrients in soils is highly heterogeneous even at fine spatial scales (Březina et al., 2019; Farley and Fitter, 1999; Jackson and Caldwell, 1993; Skálová et al., 2023) and plants explore and exploit this heterogeneity through their root systems (Giehl and von Wirén, 2014; Weiser et al., 2016). In addition, environmental nutrient heterogeneity can be explored through clonal growth, which adds another level of plant modularity. Clonal plants, which grow laterally and form multiple rooting points along their horizontal stems, are able to acquire mineral nutrients at different sites and translocate them throughout the plant body (Alpert, 1991; de Kroon et al., 1998; Noble and Marshall, 1983). In contrast to the transport of nutrients between roots and shoots, this translocation is not inevitable because the rooting units (hereafter ramets) are potentially independent. Consequently, the extent of such clonal integration varies both between and within species (Alpert, 1999; Si et al., 2020; Zhang et al., 2022).

Nitrogen is the most abundant mineral nutrient in plant tissues and is essential for many metabolic processes, including photosynthesis and nutrient uptake. In a non-clonal plant or a ramet of a clonal plant, nitrogen uptake, assimilation and distribution are regulated in a complex way and generally determined by source-sink relationships (Tegeder and Masclaux-Daubresse, 2018). In a clonal plant with multiple ramets, such regulation and nitrogen distribution likely depend on level of ramet integration. In a highly integrated clonal plant, the nitrogen translocation may also be primarily determined by source-sink relationships affected by environmental heterogeneity (Evans, 1991) and ramet size (Dong et al., 2015). Alternatively, nitrogen translocation may be unidirectional from older to younger parts (i.e. acropetal; Slade and Hutchings, 1987), possibly due to hormonal control (Alpert et al., 2002), or it may be generally limited. Across species or genotypes, translocation from younger to older parts (i.e. basipetal) seems to be the most variable (Evans, 1991; Lotscher and Hay, 1997; Slade and Hutchings, 1987, Duchoslavová and Jansa, unpubl.).

In natural environments, nitrogen is often a limiting factor for plant growth, and its availability is a major determinant of community composition and level of competition (Baer et al., 2004; Clark et al., 2007; Gough et al., 2012). Plants adapt to experienced nitrogen availability by adjusting the economy of its uptake, processing and conservation (Vázquez De

Aldana and Berendse, 1997). It is therefore an important component of a plant economics spectrum ranging from species adapted to low resource levels, focusing on resource conservation, to species of highly productive habitats, focusing on rapid resource acquisition and competition (Reich, 2014). In clonal plants, patterns of nitrogen translocation between ramets may be part of plant nitrogen economics, and, as such, may also be related to the typical availability of nitrogen. Accordingly, the benefits of resource sharing were predicted to depend on environmental conditions (Gardner and Mangel, 1999; Hutchings and Price, 1993; Mágori and Oborny, 2003).

In nutrient-poor habitats, extensive nutrient sharing to balance resource availability may be particularly important to maintain established ramets and to capture soil resources from a larger area, analogous to the 'conservation' end of the plant economics spectrum. Therefore, basipetal nutrient translocation may be beneficial in such environments where resource availability is generally low and unpredictable (Evans, 1991). On the other hand, nutrient sharing between established ramets may not be beneficial in productive habitats where mineral nutrients are not limiting and competition for light is the main determinant of plant growth. Under such conditions, local nutrient availability may be used for rapid local growth and little nutrient sharing between established ramets may occur, analogous to the 'acquisition' end of the plant economics spectrum.

I aimed to test the proposed nutrient sharing strategies on translocation of nitrogen in six species that form aboveground horizontal stems and occur in habitats of varying productivity. Growth experiments examining the effect of translocation between ramets usually integrate the effect over a longer growth period and therefore do not separate the effect of early acropetal support and translocation between established ramets. I therefore chose the stable isotope labelling approach, which allows translocation to be examined in a short period of time during at a later stage of ramet development. Specifically, I hypothesised that (i) there would be no directional nitrogen translocation under homogeneous conditions, and (ii) species from nutrient-poor habitats would translocate nitrogen basipetally to ramets with lower nutrient availability, whereas species from productive habitats would not translocate nitrogen between established ramets.

Methods

I used six stoloniferous species of non-forest habitats from different families, covering a gradient of habitat productivity measured by mean height of surrounding vegetation in

database plots and Ellenberg's N (Table 1; Chytrý et al., 2018; Herben et al., 2016). I grew each plant in a pair of 2 L pots, with the older part of the clone in one pot and the younger part in the other. In some species, these parts corresponded to clearly defined rooting ramets (*Hieracium bauhini*, *Fragaria viridis*, *Ranunculus repens*, *Potentilla reptans*), in others such a distinction was not possible due to the growth pattern of the species forming creeping monopodial stems with axillary inflorescences (*Veronica officinalis*, *Trifolium repens*). In all cases, the older and younger parts are hereafter referred to as mothers and daughters.

Plant material originated from four genotypes per species, collected in the field (Central Bohemia, the Czech Republic). The mothers were potted in mid-June 2018 and dead plants were replaced till mid-July. Mothers were watered by solution of tap water and liquid NPK fertilizer (0.01 % Wuxal with 0.008 g N/l) in this preparation period. Rooting of daughters was initiated approximately 6 weeks after the planting of the mothers. The experimental nutrient regime was set at the time of initiation of daughter rooting. I used two nutrient treatments - 'homogeneously poor' and 'poor to rich' (0.025 % Wuxal with 0.02 g N/l for poor and 0.1 % Wuxal with 0.08 g N/l for rich conditions, Fig. 1).

Table 7 The species used in the experiment ordered according to mean height of surrounding vegetation in database plots.

Species	Family	Mean height of surrounding vegetation [m]	Ellenberg's N
Hieracium bauhini	Asteraceae	0.371	1
Veronica officinalis	Plantaginaceae	0.387	4
Fragaria viridis	Rosaceae	0.395	3
Trifolium repens	Fabaceae	0.408	6
Ranunculus repens	Ranunculaceae	0.490	7
Potentilla reptans	Rosaceae	0.497	5

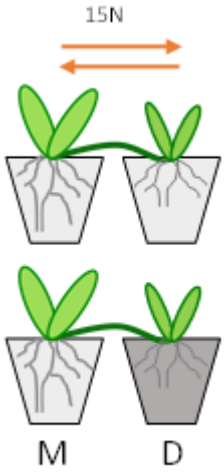

Figure 1 *Experimental setup. Plants grew either in homogeneously poor or poor to rich conditions. Light colour of pot filling depicts low nutrient regime (0.02 % Wuxal with 0.016 g N/l), dark colour depicts rich nutrient regime (0.1 % Wuxal with 0.08 g N/l). The arrows indicate tracing of ^{15}N translocation in both directions.*

Labelling and analyses

One month after daughter rooting initiation, I traced N translocation in both directions in each species and treatment, with four replicates for each combination of species, treatment, and direction. One extra plant per species was used for estimation of background ^{15}N concentration.

^{15}N was applied to the substrate of the pots with a syringe, at four positions per pot, 5 cm deep (total of 20 ml of $^{15}NH_4{}^{15}NO_3$ solution per pot, 0.1 g/l). In half of the plants, the label was applied to the mother part and in the other half to the daughter part in order to trace the N translocation in both directions. The other part of the plants was treated with the same amount of unlabelled NH_4NO_3 solution. The labelling was conducted in four time blocks with one day difference, with all treatments represented in each block.

Two days after labelling, plants were harvested, connection between mother and daughter part was severed, shoots and roots were separated, and roots were carefully washed. After drying to constant weight (at 65°C), the dry weight of plant parts was estimated. The plant parts were then homogenised, ground to a fine powder in a ball mill (MM200, Retsch, Haan, Germany) and subjected to elemental and isotopic analysis. The N concentration and isotopic composition were measured using an elemental analyser (Flash EA 2000) coupled with an isotope ratio mass spectrometer (Delta V Advantage, ThermoFisher Scientific, Waltham, MA, USA).

I used following calculations to estimate the amount of ^{15}N originating from pulse-labelling (i.e., excess ^{15}N). First, F-ratios were calculated as $R_S/(R_S+1)$, where R_S stands for molar isotope ratio in a sample ($^{15}N/^{14}N$). The amount of total nitrogen (C, in moles) was then calculated as

$$(1)\quad C = \frac{DW \times B}{a \times F + b \times (F-1)},$$

where DW is dry weight of a sample, B is molar concentration of nitrogen in a sample, a is equal to 14, b is equal to 15, and F stands for the F-ratio in a sample. The amount of ^{15}N originating from pulse-labelling (E, in moles) was finally calculated as

$$(2)\quad E = (F_S - F_L) \times C,$$

where F_S is F-ratio of a sample F_L is a limit F-ratio below which I cannot detect the stable-isotope enrichment with a sufficient confidence. This value was calculated from the F-ratios of the unlabelled control samples as 99th percentile of a normal distribution. Therefore, F-ratio values above this limit would come from this distribution with probability less than 1 %.

The amount of ^{15}N originating from pulse-labelling in unlabelled plant parts (roots and shoots combined) is further referred to as the amount of translocated ^{15}N.

Data analyses

I performed all the statistical analyses in the R statistical environment using linear models (R Core Team, 2023). The biomass, root-to-shoot ratio and N uptake were log transformed in order to meet the model assumptions. Nitrogen translocation was analysed using a general model for all the species and additional separate models for each species to test for differences between the two directions of translocation.

Relationship of net nitrogen translocation and habitat productivity (measured as mean height of surrounding vegetation in database plots) and relationship of net nitrogen translocation and mean relative daughter size were modelled by simple linear regressions. In addition, separate models were performed to test for the effect of relative daughter size on nitrogen translocation to daughters within each species. Nitrogen translocation was again square-root transformed to meet the model assumptions.

Results

Comparison of species growth

There were marked differences among the sizes of mothers of different species, while the sizes of daughters, although significant, did not differ to such an extent (Fig. 2a, Table 2). Consequently, the species markedly differed in relative daughter size (i.e. daughter biomass to total biomass ratio, Fig. 2b).

Nutrient regime had a significant positive effect on biomass of daughters (P=0.006), with only marginally significant differences among species (P=0.085, Fig. 3, Table 2). There was no significant effect of the nutrient regime on mother biomass (P=0.718, Table 2).

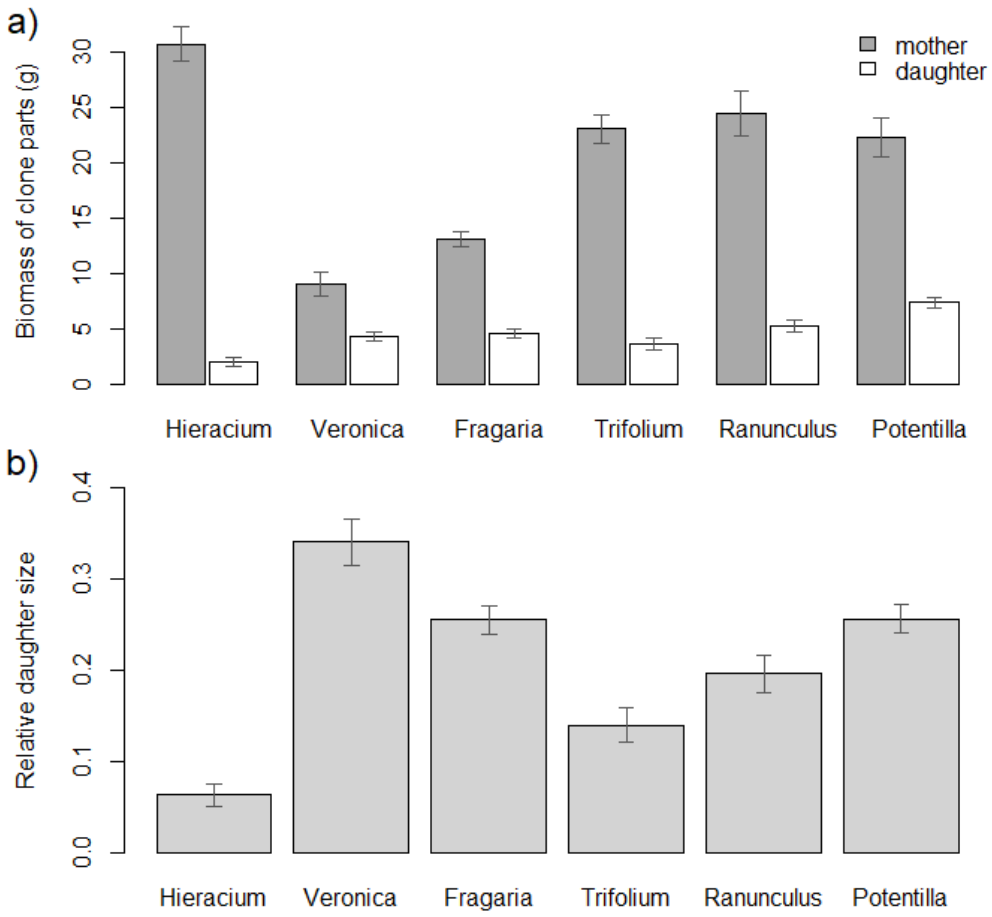

Figure 2 *a) Biomass of mothers (grey bars) and daughters (white bars) and b) relative daughter size of the six species. Relative daughter size was calculated as daughter to total biomass ratio. Means are summarized across nutrient treatments; SEMs are depicted by arrows.*

Table 2 *ANOVA of linear models of biomass (log-transformed) of different species under the two nutrient treatments. Effects with* P < *0.05 are highlighted in bold.*

Ramet biomass

		Biomass of daughters (log)			Biomass of mothers (log)		
	d.f.	Sum Sq	*F*	*P*	Sum Sq	*F*	*P*
Species	**5**	**16.64**	**18.06**	**<0.001**	**18.70**	**33.21**	**<0.001**
Nutrients	**1**	**1.45**	**7.88**	**0.006**	0.01	0.13	0.718
Species x nutrients	5	1.86	2.02	0.085	0.28	0.49	0.780
Residuals	82	15.12			9.23		

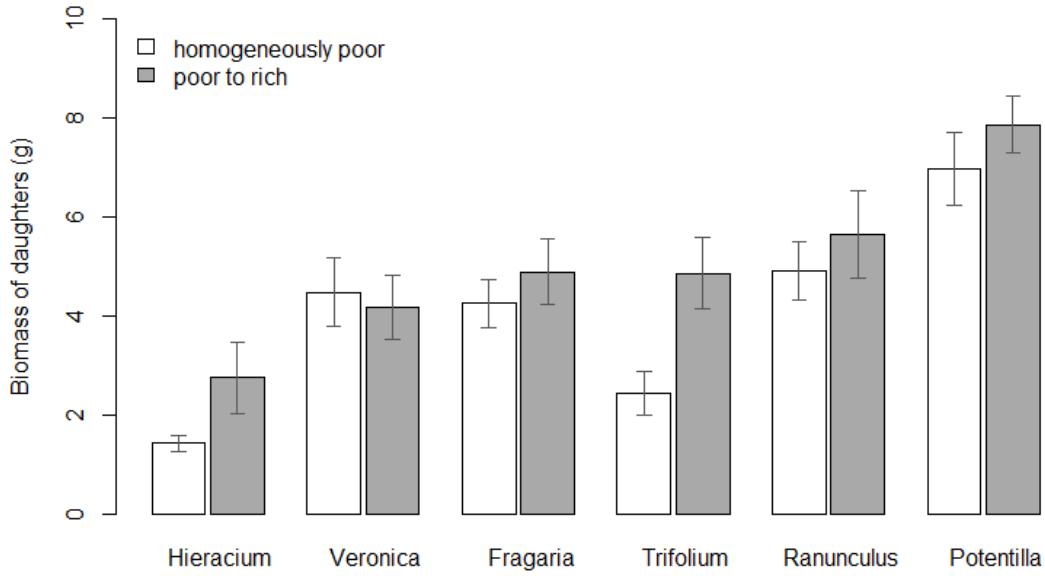

Figure 3 *Effect of nutrient treatment on biomass of daughters. Means and SEMs depicted.*

Root-to-shoot ratio significantly differed between mothers and daughters and the difference was species-specific (Table 3, Fig. 4). Whereas the root-to-shoot ratio of mothers and daughters was comparable in *Fragaria* and *Trifolium*, daughters had a markedly higher root-to-shoot ratio than mothers in *Hieracium*, and daughters had a lower root-to-shoot ratio than mothers in *Veronica*, *Ranunculus* and *Potentilla*. There was also a weak species-specific effect of nutrients on root-to-shoot ratio. Whereas most species had a neutral or negative response of root-to-shoot ratio to added nutrients, *Trifolium* had a higher root-to-shoot ratio in the poor-to-rich nutrient treatment (Fig. 4).

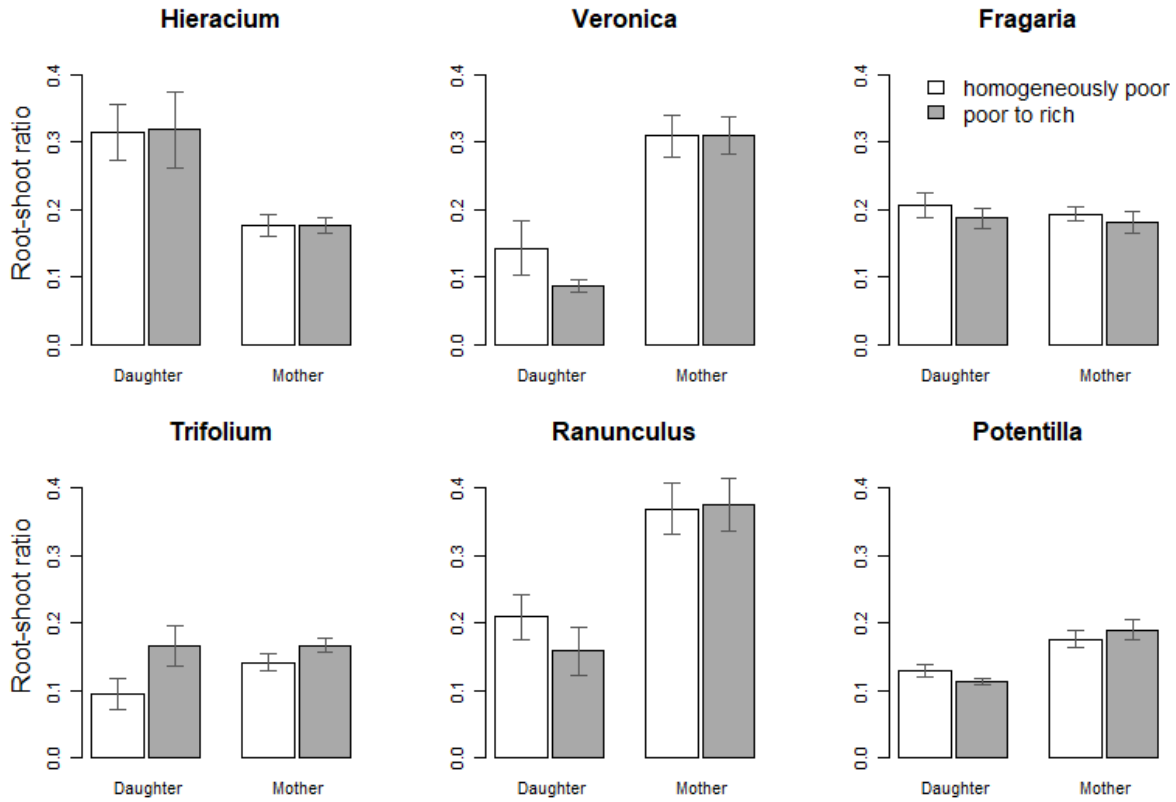

Figure 4 *Root-to-shoot ratio of daughter and mother parts in homogeneously poor (white) and poor to rich treatment (grey).*

Table 3 *ANOVA of a linear model of root-to-shoot ratio (log-transformed) of different species under the two nutrient treatments. Effects with* P < *0.05 are highlighted in bold.*

Root-shoot ratio of daughters and mothers (log)

	d.f.	Sum Sq	*F*	*P*
Species	**5**	**8.78**	**11.50**	**<0.001**
Mother/daughter	**1**	**6.48**	**42.47**	**<0.001**
Nutrients	1	0.00	0.01	0.908
Species x mother/daughter	**5**	**13.92**	**18.24**	**<0.001**
Species x nutrients	**5**	**2.20**	**2.89**	**0.016**
Mother/daughter x nutrients	1	0.10	0.64	0.426
Species x mother/daughter x nutrients	5	1.23	1.61	0.159
Residuals	164	25.03		

Nitrogen uptake

There were no marked differences in mothers' nitrogen uptake between species (Fig. 5 – y axis) or treatments. N uptake of daughters was generally lower than the uptake of mothers and it differed significantly between species, with *Veronica* uptake markedly lower than uptake of all the other species (Table 4, Fig. 5 – x axis). This could be due to the very shallow root

system of *Veronica* daughters, which may not reach the main volume of applied label. Therefore, the translocation of nitrogen to the mother may be somewhat underestimated in this species. Nitrogen treatment had no significant effect on nitrogen uptake.

Table 4 *ANOVA of a linear model of nitrogen uptake (log-transformed) of different species and mother or daughter ramets under the two nutrient treatments. Effects with P < 0.05 are highlighted in bold.*

Nitrogen uptake of ramets (log)

	d.f.	Sum Sq	*F*	*P*
Species	**5**	**8.79**	**6.80**	**<0.001**
Mother/daughter	**1**	**13.08**	**50.58**	**<0.001**
Nutrients	1	0.39	1.49	0.226
Species x mother/daughter	**5**	**13.84**	**10.71**	**<0.001**
Species x nutrients	5	1.07	0.83	0.533
Mother/daughter x nutrients	1	0.74	2.87	0.095
Species x mother/daughter x nutrients	5	0.97	0.75	0.587
Residuals	69	17.8433		

Nitrogen translocation

Direction of nitrogen translocation varied significantly between the species, with no significant effect of nutrient treatment (Table 5). Whereas *Veronica*, *Fragaria* and *Potentilla* translocated more nitrogen to daughters, *Ranunculus* and *Hieracium* translocated more nitrogen to mothers. The translocation of *Trifolium* did not differ significantly between the two directions, so net translocation was close to zero in this species (Fig. 6, Table 6).

Veronica was the only species that tended to respond to the nutrient treatment, with increased nitrogen translocation to daughters in high nutrient level (Table 6, Fig. 7).

There was no relationship between net nitrogen translocation and habitat productivity of the species (P=0.506, R^2=0.12, n=6).

Nitrogen translocation and relative daughter size

Net nitrogen translocation tended to increase with mean relative daughter size of the species (P=0.063, R^2=0.62, n=6; Fig. 7). Furthermore, there was a relationship between the nitrogen translocated to daughters and relative daughter size in *Veronica* ($P < 0.001$, R^2=0.83; Fig. 7). No such relationship was observed in the other species.

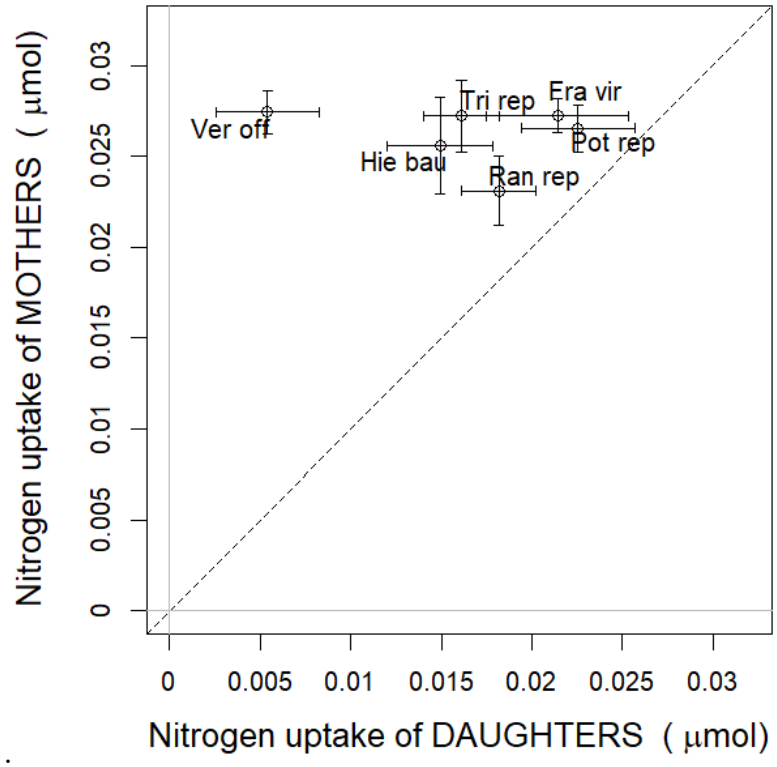

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Figure 5 *Nitrogen uptake of mothers (y axis) and daughters (x axis) of the six species. Dashed 1:1 line connects positions with equal uptake of daughters and mothers. Means and SEMs across nutrient treatments depicted.*

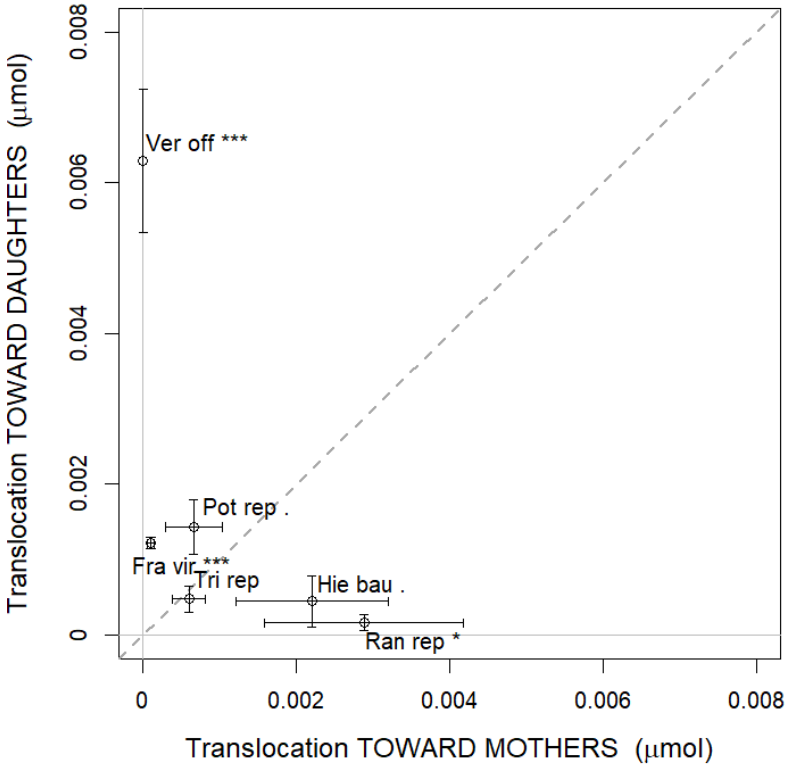

Figure 6 *Two-way translocation plot (means and SEMs). Dashed 1:1 line connects positions with equal translocation in both directions (zero net translocation). Means and SEMs across nutrient treatments depicted.*

Table 5 *Translocated nitrogen (square-root transformed) of different species under the two nutrient treatments - ANOVA of a linear model. Direction of traced translocation reflects labelling of daughters or mothers. Effects with* P *< 0.05 are highlighted in bold.*

	d.f.	Sum Sq	*F*	*P*
Species	**5**	**0.006**	**4.131**	**0.002**
Direction	**1**	**0.003**	**8.721**	**0.004**
Nutrients	1	0.000	0.160	0.690
Species x direction	**5**	**0.033**	**21.107**	**<0.001**
Species x nutrients	5	0.002	1.132	0.352
Direction x nutrients	1	0.000	1.421	0.237
Species x direction x nutrients	5	0.001	0.815	0.543
Residuals	69	0.021		

Table 6 *P-values for effects of direction and nutrients on translocation from linear models run separately for each species. Direction of traced translocation reflects labelling of daughters or mothers. Effects with* P *< 0.05 are highlighted in bold, marginally significant effects with P < 0.1 are underlined.*

	Direction	Nutrients
Hieracium	0.061	0.494
Veronica	**<0.001**	0.065
Fragaria	**<0.001**	0.149
Trifolium	0.650	0.684
Ranunculus	**0.015**	0.276
Potentilla	0.064	0.908

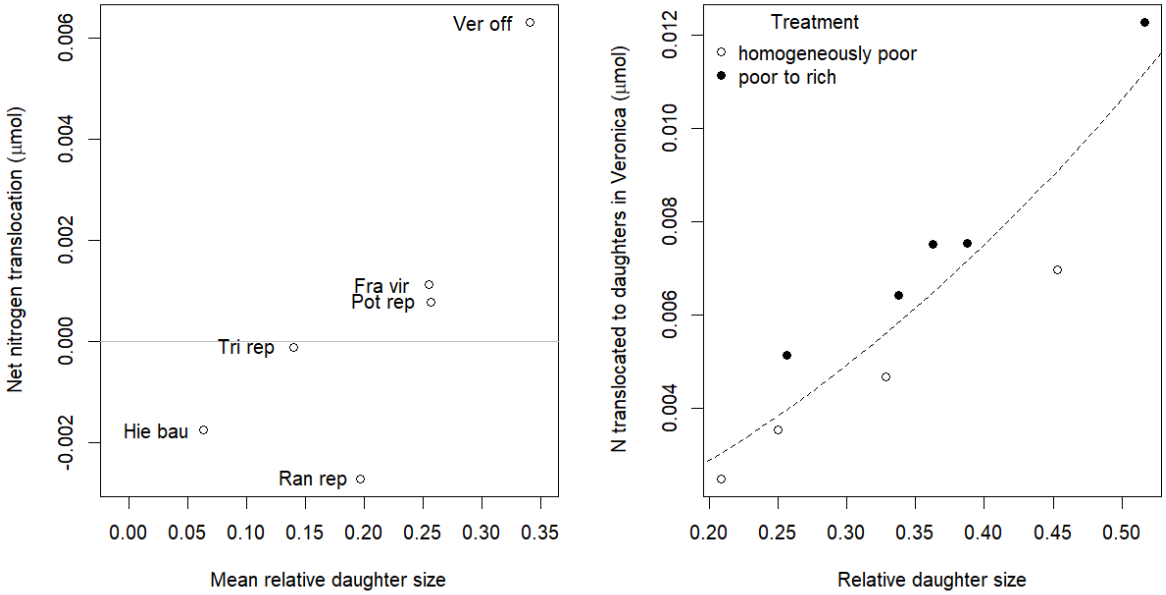

Figure 7 *Relationship of net nitrogen translocation to mean relative daughter size (left) and relationship of nitrogen translocation to daughters to relative daughter size and nutrient treatment in Veronica (right).*

Discussion

By tracing of ^{15}N in both directions, I found distinct patterns of nitrogen translocation between mothers and established daughters in the six stoloniferous species. Surprisingly, the translocation was not affected by the nutrient regime of the daughters. Irrespective of nutrient treatment, three species translocated nitrogen predominantly to daughter parts, two species to mother parts and one species showed zero net translocation of nitrogen between mothers and daughters. To our knowledge, this is the first attempt to examine translocation strategies using a comparative approach (but see e.g. Ashmun et al., 1982 or Xu et al., 2010 for two species comparisons).

I hypothesised that nitrogen would not be translocated under the homogeneous conditions and that the nitrogen sharing strategy under heterogeneous nutrient availability would reflect the productivity of the habitats typically experienced by the species. Our results did not support these hypotheses. It is possible that the number of species and the length of the productivity gradient used in our study were not sufficient to show the pattern. In general, there is a lot of variability within communities and functional groups, although there are visible patterns of plant economic strategies along environmental gradients (Maire et al., 2009).

Regardless of the environmental heterogeneity, the species studied differed greatly in their growth habit. These differences resulted in different allocations to mother and daughter parts, affecting the relative size of mother and daughter roots (i.e. sources of nutrients) and their shoots, which presumably determine the strength of nutrient sinks.

The relative allocation to acquisition of soil resources is indicated by the root-to-shoot ratio, which, in daughter ramets of stoloniferous species, inevitably increases with the development. Accordingly, the acropetal nutrient translocation is highly important for the growth of new ramets that are developing their own roots (Dong et al., 2015; Marshall, 1990). However, the root-to-shoot ratio may remain lower in daughters than in mothers in some species due to a "developmentally programmed division of labour", in which daughters specialise in the acquisition of photosynthates and mothers support them with nutrients (Roiloa, 2019; Xi et al., 2019). Therefore, prevailing translocation to daughters could be expected especially if the daughter root-to-shoot ratio is low. At the time of harvest, root-to-shoot ratio was lower in daughters than in mothers of four species, comparable between daughters and mothers in *Fragaria* and higher in daughters in *Hieracium*. However, there was no clear link between the relative root-to-shoot ratio values and net nitrogen translocation in the species.

The relative size of daughters may reflect the strength of the sink for the nutrients that they form. The results indeed indicated that net translocation of nitrogen was directed to the relatively larger daughters. This relationship was driven particularly by two of the species – by *Hieracium* forming relatively small daughters which translocate nitrogen basipetally to mothers, and by *Veronica* with relatively large daughters and high acropetal nitrogen translocation. Moreover, I observed the relationship between daughter relative size and the magnitude of translocation to daughters also at the intraspecific level in *Veronica*. Surprisingly, higher nutrient availability to daughters seemed to increase nitrogen translocation to them in this species. This pattern resembles the 'rich get richer' effect proposed in *Fragaria chiloensis* (Alpert, 1996) and *Buchloe dactyloides* (Sun et al., 2011).

Our results suggested that the growth habit of clonal species, and in particular the relative size of their ramets, is more likely to determine nutrient translocation than environmental heterogeneity in nutrient availability. The observed distinct patterns of nutrient translocation may be either an unavoidable consequence of biomass allocation or, together with biomass allocation, part of adaptive resource-sharing strategies. I hypothesise that the acropetal nitrogen translocation may facilitate exploration of new patches, whereas daughters may serve as extended hands for nitrogen acquisition in plants with the prevailing basipetal translocation (Duchoslavová and Jansa, 2018; Pinno and Wilson, 2014). Such function of daughter ramets may be temporal and serve, for example, to support flowering mother ramets by vegetative daughters, as could be the case of *Hieracium* in our experiment. In contrast to the nitrogen translocation observed here, carbon translocation seems to respond more readily to gradients in light availability and different carbon translocation strategies are not pronounced under homogeneous conditions (Duchoslavová and Jansa, 2018; Duchoslavová and Jansa, unpubl.).

More information on the net translocation of nutrients between mother and daughter parts of different clonal plants is needed to generalise the results. As growth experiments do not separate the effect of early translocation from translocation between established ramets, I encourage future studies to examine translocation of nutrients in both directions by the labelling approach, which has rarely been done to date (Dong et al., 2022; Duchoslavová and Jansa, 2018; Pinno and Wilson, 2014).

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Paper V

Effect of clonal growth form on the relative performance of species in experimental communities over time

Jana Duchoslavová[1], Tomáš Herben[1,2]

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[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, 128 43 Praha 2, Czech Republic

[2] Institute of Botany, Czech Academy of Science, 252 43, Průhonice, Czech Republic

Abstract

Due to competition for resources, the performance of plant species in communities is modified by traits affecting their efficiency in resource uptake and use. Clonal growth by stolons or rhizomes enables plants to spread laterally and to share resources among interconnected ramets; therefore, clonal growth represents an important trait that likely affects the competitive ability of species. We tested the effect of different clonal growth forms on the relative performance of plant species in communities of the Jena Biodiversity Experiment over a ten-year period. Clonal growth form did not have a significant effect on relative performance in early stages of the communities. Surprisingly, none of the clonal growth forms gained dominance over time, but the species with long rhizomes generally performed slightly better than the other species, particularly in communities of low diversity. The stoloniferous species performed better in communities with a higher proportion of nonclonals, suggesting complementarity of the light exploitation strategies of stoloniferous and nonclonal species. Our results show the importance of clonal growth traits for the competitive abilities of plants in the context of community development, species diversity and community composition, as well as the necessity of distinguishing among different clonal growth forms.

Keywords

clonal growth; competition; complementarity; rhizomes; stolons; the Jena Biodiversity Experiment

Introduction

Competition for resources is the major driver of plant community structure and affects the performance of individual plant species (Goldberg, 1990). Although all plants use similar types of resources, plant species differ in their mechanisms of resource exploration and exploitation (Schwinning and Weiner, 1998). Therefore, available resources may be used more completely by mixtures of plants with different resource-use niches (Loreau and Hector, 2001; Tilman et al., 2014). Due to these interactions, species performance in a mixed community is not a simple function of performance in a monoculture (Loreau and Hector, 2001; Roscher et al., 2011; Yu et al., 2012). The relative success of plant species in mixed communities is modified by traits affecting efficiency in the uptake and use of limited resources (Roscher et al., 2011).

We would like to highlight the fact that resource availability for plants may be altered by clonal integration, as clonal growth by stolons or rhizomes enables plants to share resources among interconnected ramets, which may benefit young ramets and established ramets growing in resource-poor patches (Song et al., 2013; Wang et al., 2016). Clonal growth thus represents another important but neglected trait that possibly affects plant interactions and performance of species in plant communities (Gross and Mittelbach, 2017; Mudrák et al., 2017; Zobel et al., 2010). Although there have been a number of experiments showing the benefits of clonal integration for individual plant fitness (for example, Roiloa et al., 2010; Song et al., 2013; You et al., 2014), little is known about the role of the clonal growth form in competitive conditions. However, the importance of the clonal growth form for community structure was demonstrated, for example, in fertilized grasslands in North America (Gross and Mittelbach, 2017, but see Peltzer, 2002; Pennings and Callaway, 2000), and species with a higher capacity for lateral expansion have been shown to have a greater local abundance in plant communities in the Czech Republic (Herben et al., 2014).

Complementarity of species and functional groups in resource use has been shown, not only to increase productivity of communities (Cardinale et al., 2007; Tilman et al., 1997) but also to affect other community properties. For example, in the Jena Biodiversity Experiment with different assemblages of temperate grassland species, higher diversity in clonal growth increased spatial stability of the experimental communities, possibly due to the complementarity of growth strategies (Weigelt et al., 2008). Therefore, clonality may be especially beneficial in communities with a low variation in growth strategies, for example, low proportion of clonal species. There are a number of functions that differentiate clonal species from nonclonal ones. In addition to resource sharing, clonal growth allows for specific ramet positioning. The wide spacing of ramets enables quick colonization and exploitation of open patches and may thus bring competitive advantages in vegetation of lower density (Lenssen et al., 2005; Schmid and Harper, 1985; Zobel et al., 2010), whereas the aggregated distribution of ramets has been shown to be advantageous in dense vegetation without open patches (Schmid and Harper, 1985) and promotes the coexistence of species by reducing the level of

interspecific competition (Bolker et al., 2003). With an increasing number of species in a community, intraspecific interactions are replaced by interspecific interactions, and vegetation may become denser (Marquard et al., 2009). Therefore, wide-spreading clonal species may benefit the most in communities with a low number of species, whereas species with a clumped ramet distribution may be most favoured in communities of high species diversity (Bolker et al., 2003). We thus expected species with different clonal growth forms to respond differently to the number of species in a community.

In addition, the benefits and costs of clonal growth in the initial developmental stages of communities that are recruited from seeds may differ from those in established vegetation. The initial growth of clonal species after germination could be slower due to their investment into clonal organs (Šmilauerová and Šmilauer, 2007, Martínková et al., in review), whereas vegetative propagation could be more efficient than recruitment from seeds in an established vegetation (Mudrák et al., 2017). Indeed, studies of the abundances of species in restored meadows indicate that in clonal species, the initial disadvantage of slow recruitment from seeds is later outweighed by ability to spread vegetatively (Albert et al., 2019; Mudrák et al., 2017). Based on this previous work, we hypothesized that clonal species would perform worse than nonclonal species in the early stage of development of communities, and that the clonal species would gain dominance later in established vegetation, especially when the proportion of clonal species in a community is low.

To test our hypotheses, we used publicly available data from temperate grassland communities of the Jena Main Experiment together with the corresponding monocultures of all the target species from the years 2003-2012 (Weigelt et al., 2016). The Jena Main Experiment was established in 2002 and involved the sowing of species mixtures in open space to study the effects of biodiversity on the functioning of plant communities. The analysed communities of the Jena Main Experiment were composed of 2, 4, 8 and 16 species out of the total pool of 60 species. Out of these 60 species, 41 species grew clonally. Although the experiment was not designed to analyse the effects of clonal growth, the communities were composed of species with different forms of clonal growth, and the proportions of species with these different growth forms differed among the communities, which allows for the testing of our hypotheses. We distinguished the following 4 growth forms with respect to clonal growth: non-clonals, species with stolons, species with long rhizomes and species with short rhizomes (Table 1). The division was based on the morphological types of clonal growth organs used in the CLO-PLA database (Klimešová et al., 2017), which are known to function differently in the field (Herben and Klimešová, 2020). We used the relative species yield (RY), which compares the species biomass in a mixture and a monoculture, as a measure of the relative success of individual species (Roscher et al., 2011). This measure emphasizes different effects of inter- and intraspecific interactions on plant performance, while it suppresses the effect of inevitable biomass differences between species of different sizes.

We addressed the following questions using RY:

i) Do species with different growth forms differ in their relative success in the early development of the communities?

ii) Does effect of growth form on relative success in communities change over time?

iii) Do the effects of growth form on species performance change with the number of species and the proportion of nonclonal species in a community?

In addition, if any of the growth forms is superior in competition, it should contribute disproportionally more to relative community yield. Relative community yield (RYT) is an attribute of whole communities and is expressed as a sum of the RYs of all species in a mixture (Inouye and Schaffer, 1981; Jolliffe et al., 1984). Therefore, we raised another question:

iv) Do species with different growth forms contribute disproportionally to relative community yield?

Finally, we wanted to test the necessity of distinguishing different clonal growth forms, as the variability of clonal growth is often neglected. Thus, we added one last question:

v) Would the conclusions substantially differ if different clonal growth forms were considered as a whole?

Materials and methods

The Jena Experiment

Experimental communities in the Jena Main Experiment were established in 2002 on 20 x 20 m plots on former arable land in a floodplain of the Jena River in Germany. These communities were sown from a pool of 60 grassland species and maintained by bi-annual mowing and weeding. The communities used for the presented analyses were composed of 2, 4, 8 or 16 species (communities with 1 and 60 species were omitted from the presented analyses as there was no variability in species composition). There were 14 plots for the 16-species communities and 16 plots for each of the other levels of sown diversity, giving a total of 62 plots included in analyses. Communities with the same species richness differed in species composition. Monocultures were established for each species in smaller plots of 3.5 x 3.5 m. There were initially 2 replicates of monocultures for each species, but one of the replicates was later omitted. The initial total plant density was held constant for all the mixture plots and for the monocultures. The biomass of the sown species was harvested and weighted twice a year. For our analyses, we used biomass data from the first harvest in each season only (typically in May), because this was when biomass was highest. See Weigelt et al. (2016) for further details of the experiment.

Table 1 *Division of the target species in the Jena Main Experiment according to their growth forms. Persistence of clonal organs (years) and clonal spread per year (m) from the CLO-PLA database and height (m) from the LEDA traitbase are provided for each species* (Kleyer et al., 2008; Klimešová et al., 2017)*.The means were calculated for each growth form.*

	Persist.	Spread	Height		Persist.	Spread	Height
Long rhizomes	3.83	0.12	0.56	**Stolons**	2.11	0.20	0.35
Achillea millefolium	4	0.14	0.32	*Ajuga reptans*	2.4	0.19	0.20
Arrhenatherum elatius	4	0.08	0.60	*Glechoma hederacea*	1.7	0.37	0.60
Avenula pubescens	4	0.08	0.50	*Poa trivialis*	3	0.07	*NA*
Centaurea jacea	3.3	0.04	0.85	*Prunella vulgaris*	2.1	0.13	0.18
Festuca rubra	4	0.07	0.10	*Ranunculus repens*	1.2	0.25	0.38
Galium mollugo	3.6	0.17	0.50	*Trifolium fragiferum*	1.7	0.17	0.30
Lathyrus pratensis	3.8	0.21	0.75	*Trifolium repens*	1.9	0.27	0.50
Luzula campestris	3.6	0.08	0.38	*Veronica chamaedrys*	2.9	0.13	0.30
Poa pratensis	4	0.07	0.30				
Vicia cracca	4	0.22	1.30				
Short rhizomes	3.61	0.04	0.41	**Nonclonal**	*NA*	*NA*	0.45
Alopecurus pratensis	4	0.05	0.45	*Bromus hordeaceus*			0.07
Anthoxanthum odoratum	3.6	0.03	0.17	*Campanula patula*			0.40
Anthriscus sylvestris	3.2	0.01	0.80	*Carum carvi*			0.45
Bellis perennis	2.7	0.10	0.06	*Crepis biennis*			0.58
Bromus erectus	4	0.01	0.50	*Daucus carota*			0.10
Cardamine pratensis	3	0.11	0.25	*Heracleum sphondylium*			1.25
Cirsium oleraceum	3.3	0.09	0.80	*Knautia arvensis*			0.63
Cynosurus cristatus	4	0.01	0.40	*Lotus corniculatus*			0.10
Dactylis glomerata	3.6	0.04	0.13	*Medicago lupulina*			0.33
Festuca pratensis	4	0.04	0.55	*Medicago x varia*			*NA*
Geranium pratense	4	0.01	0.55	*Onobrychis viciifolia*			0.35
Holcus lanatus	4	0.05	0.33	*Pastinaca sativa*			1.05
Leontodon autumnalis	3	0.01	0.11	*Pimpinella major*			0.85
Leontodon hispidus	3.1	0.02	0.24	*Taraxacum officinale*			0.20
Leucanthemum vulgare	4	0.13	0.33	*Tragopogon pratensis*			0.35
Phleum pratense	4	0.01	0.16	*Trifolium campestre*			0.35
Plantago lanceolata	*NA*	*NA*	0.07	*Trifolium dubium*			0.25
Plantago media	*NA*	*NA*	0.43	*Trifolium hybridum*			0.30
Primula veris	4	0.03	0.20	*Trifolium pratense*			0.53
Ranunculus acris	2.75	0.02	0.58				
Rumex acetosa	3.7	0.03	1.00				
Sanguisorba officinalis	4	0.03	0.65				
Trisetum flavescens	4	0.07	0.75				

Division of growth forms

We divided the species into four groups (hereafter called "growth forms") based on morphological types of clonal growth organs in the CLO-PLA database (Klimešová et al., 2017). The four growth forms are as follows: (i) clonal species with hypogeogenous rhizomes, i.e., relatively persistent and fast-spreading belowground connections between shoots ("long rhizomes"); (ii) clonal species with epigeogenous rhizomes, i.e., relatively persistent and slow-spreading belowground connections between shoots ("short rhizomes"); (iii) species with stolons, i.e., relatively fast-spreading but rather short-lived aboveground connections ("stolons"); and (iv) species without clonal propagation ("nonclonals", Table 1). *Plantago lanceolata* and *Plantago media* were added to the short rhizomes group on the basis of personal observations (Christiane Roscher, personal communication). This division of species combines the potential for the integration of ramets with the potential speed of lateral spread.

Relative performance of individual species

As a measure of the relative performance of the individual species in mixtures, we used relative yield (RY, Roscher et al., 2011).

$$RY = \ln\left(\frac{biomass\ in\ mixture\ \times species\ number}{biomass\ in\ monoculture}\right)$$

The biomass was estimated per unit area; multiplication by species number accounted for different sown densities of the species in mixtures and monocultures, since total sown density was the same in all plots. A value above zero indicated a greater biomass of a species in the mixtures than expected from monocultures, whereas values below zero indicated a smaller biomass in the mixtures than expected from monocultures.

To describe the properties of each plot in our models, we used the number of sown species and proportion of sown nonclonal species in a plot. The species for which a given RY was calculated was not taken into account in the calculation of proportion of nonclonals. The species number and proportion of nonclonal species in plots were not independent of each other, as the experiment was not designed for such analyses. The proportion of nonclonals slightly declined with the number of sown species (R^2=0.04, P<0.001). However, excluding one of the variables from the models did not qualitatively change the estimated effect of the other variable.

We analysed the effects of the species and plot properties on RY using linear mixed-effects models to describe the hierarchical structure of the data (lme4 package, Bates et al., n.d.) in the R environment (R Core Team, 2016). Individual observations in the models were presented as measures of a single species in a specific plot and year, yielding a total of 3025 non-missing observations with the hierarchical structure given by the species assemblage of the communities. To filter out patterns

caused by phylogenetic relationships between species, we used phylogenetic eigenvectors following the models of Diniz-Filho et al. (1998) for the performance of individual species. We used 11 eigenvectors explaining 91 % of the phylogenetic variability in the data as a covariate in the models. We calculated p-values based on the Satterthwaite approximation of the degrees of freedom using the lmerTest package (Kuznetsova et al., 2017). We inspected the residuals of all models for potential heteroscedasticity across the covariates. We also identified outliers based on Cook's distance and dropped them from a model if needed. To determine significant differences between modelled slopes, we used the P_t values of the estimates of slope differences using treatment contrasts with nonclonals as a reference level.

To answer the first question, we analysed the initial performance of the species using biomass data from 2003, i.e., one year after sowing. Growth form, sown species number and nonclonal species proportion were included in the model as fixed effects, and we allowed for two-way interactions of all the fixed effects. All predictors were scaled (mean-centred). To describe the hierarchical structure of the data, the species identity and plot identity were included as crossed random effects affecting the intercept.

We used data from 2003-2012 to answer the second and third questions. The structure of fixed effects in the model was the same as above with the time included as a linear and quadratic fixed effect to account for potential nonlinearity. Species identity and plot identity were again included as crossed random effects affecting the intercept, and the effects of both were allowed to change with time.

In addition, we assessed if the observed patterns in RY were driven by the differential growth of species with different growth forms in monocultures or mixtures. We thus repeated the previous analyses using absolute biomass in the mixtures corrected for sown diversity (to account for different initial density of species in plots with different species richness) as a response variable. The structure of the fixed and random effects was the same as in the previous models. We also added a model of biomass in the monocultures with the same structure. The biomass was log-transformed in all models to meet the model assumptions.

To assess the necessity of distinguishing different forms of clonal growth (fifth question), we repeated the RY analyses with all species with clonal growth merged into a single category, yielding a two-level trait (i.e., clonals vs. nonclonals).

Contribution of the species with different growth forms to relative community yield

We used the relative yield total (RYT) as a measure of community yield (Inouye and Schaffer, 1981; Jolliffe et al., 1984). RYT is the sum of the relative yields of all species in a mixture, and it is independent of sown diversity. Additionally, we calculated the contribution of the four growth forms to RYT. Therefore,

$RYT = \mathrm{RYT1} + \mathrm{RYT2} + \mathrm{RYT3} + \mathrm{RYT4}$,

where RYT is relative yield total, and the RYT1 to RYT4 values are the sums of the relative yields of the four growth forms. Consequently, RYT1/RYT to RYT4/RYT are relative contributions of the particular growth forms to RYT. If all the growth forms establish and grow equally, the expected relative contribution of a growth form to RYT would be equal to its sown proportion in a community. Therefore, when a relative contribution to RYT differs from the expectation, the difference of the relative contribution to RYT and sown proportion in a community differs from zero. Hereafter, we refer to this difference as "deviation from the proportional contribution to RYT".

To answer the fourth question, we analysed the deviation from the proportional contribution of the growth forms to RYT using separate linear mixed-effect models for each growth form. We used the difference between relative contribution to RYT and the sown proportion of the growth form in a mixture as a response variable. We included the number of species with non-zero biomass, time as a linear and quadratic term and the proportion of the particular growth form (based on species with non-zero biomass) as predictors with fixed effects. We scaled all the fixed effects to make effect sizes comparable and eliminate correlation of the intercept and slope estimates. Time and plot identity were included as random effects. In these models, a non-zero intercept (obtained from the model coefficient estimates) indicates overall deviation from the expected proportional contribution of a growth form to RYT, whereas a non-zero slope indicates response of this deviation to predictors. The use of the intercept estimate in the interpretation is needed here, as the main question includes the difference of the response variable from zero (i.e., the expected value). We excluded communities with only a single growth form and communities without the growth form in the question from the analyses.

It is important to note that these models are not fully independent of each other, as the four relative contributions in a community sums up to one. However, separate univariate linear models enabled us to detect non-zero intercepts (i.e., deviation from the expected relative contributions). Similar models with clonality as a binary trait would be fully dependent on each other (correlation coefficient of response variables equals -1) and are not applicable.

Results

The relative performance of species

One year after sowing, the relative yield of the four growth forms did not differ significantly (Table 2). However, the relative yield of species with different growth forms changed differently over time (Table 3), with the relative performance of nonclonals growing linearly, and the performance of all the clonal groups showing a humped shape (i.e., the quadratic coefficient of time did not significantly differ from zero for nonclonals, while for all the clonal groups, it was significantly lower than for nonclonals, Figure 1). Species with different growth forms responded differently to both sown species

number and nonclonal proportion in the community (Figure 2, interaction terms in Table 3). The relative performance of nonclonals slightly increased with sown species number. This effect was initially nonsignificant, but it significantly increased over time (estimated effects of species number and the interaction of species number and linear term of time are equal to 0.11 and 4.95, respectively, with $P_t = 0.20$ and 0.02, respectively). Only the species with long rhizomes differed significantly from the nonclonals, by responding more negatively to the sown species number. This phenomenon resulted in a higher relative performance of the species with long rhizomes in low-diversity plots and similar relative performances of all the growth forms in high-diversity plots (Figure 2 A). The proportion of nonclonals in plots did not significantly affect the RY of the nonclonals and species with long or short rhizomes, but the stoloniferous species performed significantly better in plots with a higher proportion of nonclonals (Figure 2 B).

Biomass

The biomass in the monocultures declined with time in all 4 growth forms and did not significantly differ among the growth forms (Table S1, Figure S1 in the Supplementary material). There were no significant effects of the predictors on biomass in mixtures one year after sowing. In the analysis of biomass in mixtures over time, the significance and direction of the effects were very similar to those in the analysis of RY, except that the interaction of growth form and time was not significant (Table S1, Figure S1).

Clonality as a single trait

When the models were rerun with growth form as a binary trait, the only differences observed between the clonals and nonclonals were in the change in RY over time (with the RY of the nonclonals growing linearly and the RY of the clonals showing a humped shape). The interactions of growth form with sown species number and nonclonal proportion were not significant (Table S2, Figure S2). The Akaike information criterion was lower for the full model with 4 growth forms than for the model with 2 growth forms (difference equals 11).

Contribution of species with different growth forms to the relative community yield

As indicated by nonzero intercepts, contribution to RYT was significantly higher than expected in the species with long rhizomes and lower than expected in the species with short rhizomes and in the stoloniferous species. Contribution to RYT did not significantly differ from expected values in the nonclonals (Table 4, Figure 3). Furthermore, there was a significant change in the contribution of the nonclonals and stoloniferous species to RYT with time, with the stoloniferous species contributing more and the nonclonals contributing less to RYT in the middle of the observed period, which was approximately 6 years after sowing (Table 4, Figure 3).

Table 2 *Analysis of RY one year after sowing (2003) – ANOVA table of type II with the Satterthwaite approximation for denominator degrees of freedom (DenDF). Significant effects (P_F<0.05) are depicted in bold.*

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)
Phylogeny	**29.078**	**2.643**	**11**	**40.855**	**2.526**	**0.016**
Growth form (F)	3.330	1.110	3	38.679	1.061	0.377
Sown diversity (S)	0.150	0.150	1	55.450	0.143	0.707
Proportion of nonclonals (NC)	1.570	1.570	1	84.351	1.500	0.224
F x S	2.400	0.800	3	272.276	0.765	0.515
F x NC	3.136	1.045	3	256.725	0.999	0.394
NC x S	0.002	0.002	1	67.940	0.002	0.966

Table 3 *Analysis of RY over time (2003-2012) – ANOVA table of type II with the Satterthwaite approximation for denominator degrees of freedom (DenDF). Significant effects (P_F<0.05) are depicted in bold.*

	Sum Sq	Mean Sq	NumDF	DenDF	F.value	Pr(>F)
Phylogeny	48.94	4.45	11	46.61	1.77	0.088
Sown diversity (S)	1.85	1.85	1	57.42	0.73	0.395
Proportion of nonclonals (NC)	2.55	2.55	1	68.91	1.01	0.317
Growth form (F)	12.97	4.32	3	44.92	1.72	0.177
Time	**64.07**	**82.04**	**2**	**65.78**	**32.60**	**<0.001**
S:NC	0.06	0.06	1	76.96	0.02	0.876
S:F	**25.44**	**8.48**	**3**	**1287.39**	**3.37**	**0.018**
S:time	**17.05**	**8.53**	**2**	**117.52**	**3.39**	**0.037**
NC:F	**49.56**	**16.52**	**3**	**1173.91**	**6.56**	**<0.001**
NC:time	3.51	1.76	2	369.99	0.70	0.499
F:time	**67.43**	**11.24**	**6**	**96.51**	**4.47**	**<0.001**

[1] Time was included in the model as a second-degree polynomial, and its contribution to explained variability is thus specified on a single line. Based on the comparison of the nested models, the quadratic term was highly significant (P_{Chisq} < 0.001).

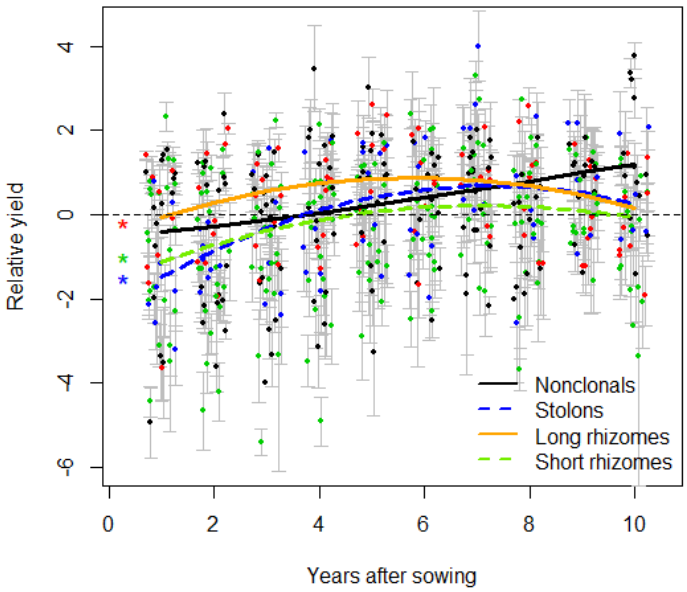

Figure 1 *Relative yield of different clonal growth forms in response to time from sowing (jittered along the x axis). Data points with error bars indicate the means and SE for a particular species and predictor level. Values of RY above zero (dashed line) indicate a greater biomass of a species in mixtures than expected from monocultures in a respective year, whereas values below zero indicate a smaller biomass in mixtures than expected from monocultures. Asterisks indicate growth forms with a significant difference in their response to time from the reference nonclonal group ($P_t < 0.05$).*

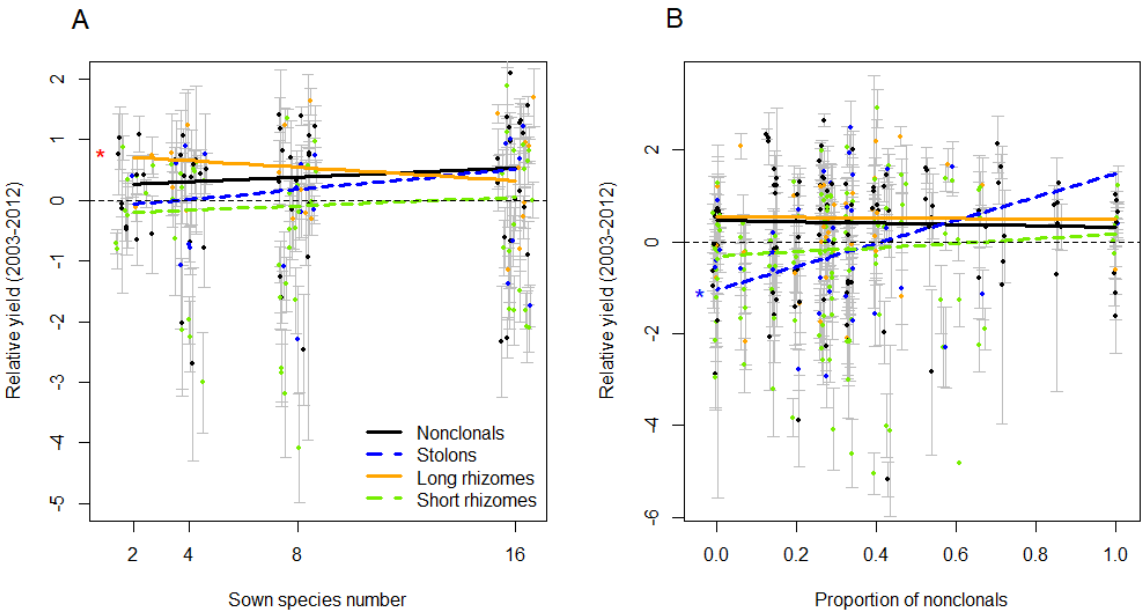

Figure 2 *Relative yield of different clonal growth forms (jittered along the x axis) in response to sown species number (A) and sown nonclonal proportion (B) over the ten-year period. Data from all the years are combined in the figures. Data points with error bars indicate the means and SE for a particular species and predictor level. Values of RY above zero (dashed line) indicate a greater biomass of a species in mixtures than expected from monocultures, whereas values below zero indicate a smaller biomass in mixtures than expected from monocultures. Asterisks indicate growth forms with a significant difference in their response to a depicted predictor from the reference nonclonal group ($P_t < 0.05$).*

Table 4 *Analyses of the deviation from the expected proportional contribution of the growth forms to RYT - ANOVA tables of type II with the Satterthwaite approximation for denominator degrees of freedom (DenDF). Significant non-zero intercept estimates ($P_t<0.05$, in bold) indicate the overall deviation from the expected proportional relative contribution to RYT, whereas significant response of the deviation to the predictors is indicated by the significant contribution of the predictors to explained variability ($P_F<0.05$, in bold).*

	NONCLONALS				
	Estimate	Std. Error	d.f.	t value	Pr(>|t|)
intercept	0.026	0.024	42.6	1.107	0.274
	Sum Sq	d.f.	DenDF	F.value	Pr(>F)
species number	0.006	1	55.3	0.134	0.715
time	**0.605**	**2**	**74.8**	**6.881**	**0.002**
proportion in community	0.027	1	67.7	0.610	0.437
	SHORT RHIZOMES				
	Estimate	Std. Error	d.f.	t value	Pr(>|t|)
intercept	**-0.044**	**0.021**	**46.5**	**-2.092**	**0.042**
	Sum Sq	d.f.	DenDF	F.value	Pr(>F)
species number	0.012	1	52.4	0.282	0.598
time	0.010	2	76.9	0.119	0.888
proportion in community	0.056	1	69.3	1.283	0.261
	LONG RHIZOMES				
	Estimate	Std. Error	d.f.	t value	Pr(>|t|)
intercept	**0.072**	**0.020**	**28.03**	**3.596**	**0.001**
	Sum Sq	d.f.	DenDF	F.value	Pr(>F)
species number	0.026	1	33.6	0.742	0.395
time	0.105	2	51.6	1.489	0.235
proportion in community	0.042	1	42.0	1.174	0.285
	STOLONS				
	Estimate	Std. Error	d.f.	t value	Pr(>|t|)
intercept	**-0.042**	**0.017**	**30.9**	**-2.521**	**0.017**
	Sum Sq	d.f.	DenDF	F.value	Pr(>F)
species number	0.061	1	32.4	1.612	0.213
time	**0.490**	**2**	**47.9**	**6.532**	**0.003**
proportion in community	0.000	1	33.8	0.012	0.914

[1] Time was included in the models as a polynomial of degree 2, and its contribution to explained variability is thus specified on a single line. Based on the comparison of the nested models, the quadratic terms in the models with the stoloniferous and nonclonal species were significant (P_{Chisq} = 0.002 and P_{Chisq} < 0.001, respectively).

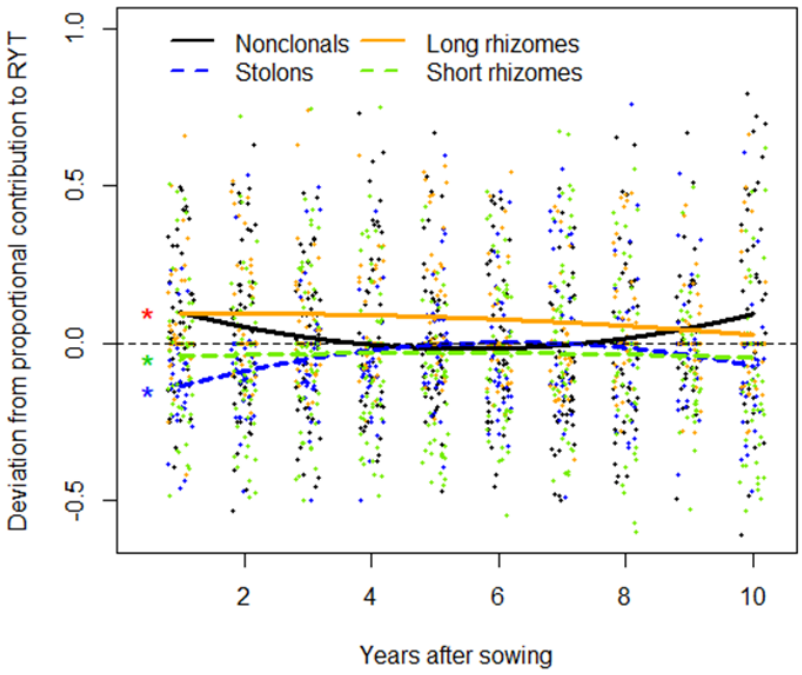

Figure 3 *Deviation from the expected proportional contribution of clonal growth forms to RYT in response to time (values jittered along the x axis). Values above zero indicate a greater contribution of the respective clonal growth form to the relative yield of the community than expected from the sown proportions, whereas values below zero indicate a smaller contribution than expected. Asterisks indicate significant deviations from the expected zero value as indicated by the non-zero intercepts (Pt < 0.05).*

Discussion

We examined the effect of clonal growth form on the relative success of plant species in the experimental communities of the Jena Main Experiment over a ten-year period. We expected the clonal plants to be disadvantaged in the early development stage of communities after sowing due to initial investments in clonal organs. In previous studies, species with more extensive clonal growth grew more slowly after germination and were more susceptible to early disturbance, likely due to their initial investment in clonal organs (Albert et al., 2019; Šmilauerová and Šmilauer, 2007, Martínková et al., in review). However, we found no effect of growth form on the relative performance of species in the first year after sowing. Therefore, if clonal growth produced disadvantages for plants in the early stage of community development, this effect was not pronounced.

We also expected the initial disadvantage of clonal species to be compensated later in community development due to their ability to spread laterally and share resources among ramets. Surprisingly, none of the growth forms gained clear dominance during the first ten years in the communities of the Jena Main Experiment. Instead, the relative yield of all the growth forms grew with time showing different shapes of the response curves. The RY of the nonclonals increased linearly, but the RY of the three clonal groups showed a humped shape with a slight decline at the end of the observed period. Humped shape of the relationship was maintained when clonal growth forms were merged and seems

to be caused mainly by different processes in monocultures and mixtures at the end of the ten-year period. Whereas absolute biomass of clonal species in mixtures slightly declined, it remained stable or increased in monocultures. Consequently, the relative yield of the clonal growth forms declined at the very end of the period whereas the relative yield of nonclonals increased. The overall linear increase in the RY was in accordance with the known increase in overyielding over time, and seems to be caused by a stronger decline in the average biomass of the monocultures than in the mixtures (Marquard et al., 2013), at least in the beginning of the studied period. This observation is supported by the fact that the absolute biomass of any growth form in mixtures did not increase with time. Additionally, the contributions of the growth forms to relative community yield did not show increasing dominance of any growth form with time. These results did not provide evidence for the hypothesized increase of benefits of extensive clonal growth with community development.

The species with long rhizomes did perform slightly better than the other growth forms across the studied period. Although the general effect of clonal growth form on the relative performance of individual species was not pronounced, the RY of the species with long rhizomes was higher than the RY of the other growth forms in communities of low diversity. Similar results were observed for the biomass of the species with long rhizomes. In addition, only the species with long rhizomes generally contributed more to the total relative yield of communities than expected on the basis of their sown proportion. This finding is in accordance with the expected advantage of long and persistent connections between ramets in vegetation of low diversity, which was accompanied by a lower shoot density in the Jena experiment (Marquard et al., 2009). When shoot density is low, relatively fast-spreading long-rhizome species may colonize space effectively. High neighbour diversity and higher shoot density possibly do not leave enough space for expansion of the long rhizome species (Fahrig et al., 1994). Moreover, neighbour diversity may affect the scale of spatial heterogeneity, which has been shown to influence competitive benefits of clonal integration (Eilts et al., 2011). Thus, spatial heterogeneity generated by a low number of species may be of optimal grain size for foraging by long rhizomes. In contrast, there was no indication of the expected benefits of species with short rhizomes at high species diversity. Instead, the species with short rhizomes showed generally low relative performance and lower than proportional contribution to the relative yield of the community. The short rhizome species thus performed best in monocultures, and they were inferior in communities where interspecific interactions prevailed. We expected these species to be favoured in dense vegetation without open patches, which do not seem to be present in the Jena communities.

Plants with clonal growth forms have been expected to be at an advantage in communities with a prevalence of nonclonal species due to the presence of incompletely filled clonal growth niches. Here we show that in communities with a low proportion of nonclonals, unfilled clonal growth niches may not exist, and plants with clonal growth may be at a disadvantage. However, we found that high proportion of nonclonals in a community only had a positive effect on the performance of the

stoloniferous species. Therefore, clonal growth by stolons seems to be efficient in the absence of other clonal species and possibly disadvantageous in pure clonal communities. This might be caused by complementary light exploitation strategies of the stoloniferous and nonclonal species, as stoloniferous species are generally of low stature and forage for light by lateral growth (Dong and Pierdominici, 1995; Gruntman et al., 2017; Macek and Lepš, 2003). Diversity in clonal growth forms has been previously shown to be important for the spatial stability of communities in the Jena Experiment (Weigelt et al., 2008).

When we compared clonal and nonclonal species without distinguishing the different clonal growth forms, no effects of the number of sown species and the proportion of clonals on RY were observed. The previously significant effects diminished because species with different clonal strategies responded differently to the community properties. Therefore, studies using clonality as a single category may neglect interesting patterns because important differences among clonal strategies are not taken into account. Recognizing different clonal growth forms is thus essential for understanding the role of clonal growth in the functioning of plant communities.

Conclusions

Our results did not confirm the expected disadvantage of clonal growth in the early development stage of communities after sowing. Contrary to our expectation, none of the growth forms gained clear dominance during the studied ten-year period; however, the species with long rhizomes did perform slightly better than other species, at least in species-poor communities. Furthermore, we found evidence for the complementarity of nonclonal and stoloniferous growth strategies, as the relative performance of species with stolons was higher in nonclonal communities. Therefore, our findings highlight the importance of clonal growth traits for the plant community structure and competitive ability of plant species in the context of community composition and species richness. However, they also imply that the primary mechanism of coexistence of clonal and nonclonal species in grassland communities is not strong niche differentiation between clonal and nonclonal species, but is at least partly driven by neutral processes, possibly due to different spatial dynamics of each of these growth forms. Consequently, our findings also support the notion that different clonal growth strategies show different long-term dynamics and therefore should be treated separately in ecological analyses.

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