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With a little help from my friends: physiological integration facilitates invasion of wetland grass *Elymus athericus* into flooded soils

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Tidal wetlands worldwide are undergoing rapid invasions by tall-growing clonal grasses. Prominent examples are invasions by species of the genera *Spartina*, *Phragmites* and *Elymus*. The responsible physiological and ecological drivers of these invasions are poorly understood. Physiological integration (PI) is a key trait of clonal plants, which enables the exchange of resources among ramets. We investigated PI in *Elymus athericus*, which has been rapidly spreading from high-marsh into low-marsh environments of European salt marshes during the last decades. We applied a nitrogen stable-isotope approach to trace nutrient translocation between ramets in a factorial mesocosm experiment. The experiment was set up to mimic an invasion pattern commonly found in tidal wetlands, i.e. from high-elevated and rarely flooded into low-elevated and frequently flooded microenvironments. We tested for intraspecific variability in PI by including two genotypes of *Elymus* that naturally occur at different elevations within the tidal frame, a high-marsh (HM) and a low-marsh (LM) genotype. PI strongly increased offspring ramet aboveground and belowground biomass by 62 and 81%, respectively. Offspring ramets under drained conditions had 95% greater belowground biomass than those under flooded conditions. LM genotype offspring ramets produced 27% more aboveground biomass than HM genotypes. Offspring ramets were clearly more enriched in ^{15}N under flooded versus drained conditions; however, this positive effect of flooding on $\delta^{15}N$ was only significant in the LM genotype. Our findings demonstrate the importance of PI for the growth of *Elymus* offspring ramets and thereby for the species' capacity for fast vegetative spread. We show that offspring ramets under stressful flooded conditions are more dependent on nutrient supply from parent ramets than those under drained conditions. Our data furthermore suggest a higher degree of adaptation to flooding via PI in the LM versus HM genotype. In conclusion, we highlight the importance of assessing PI and intraspecific trait variability to understand invasion processes within ecosystems.

Keywords: clonal integration, genotype–environment interaction, intraspecific variability, ^{15}N tracing, range expansion, Wadden Sea

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Introduction

Physiological integration (PI) is a key trait of clonal plant species, enabling them to share essential resources (i.e. nutrients, assimilates and water) among a network of interconnected ramets (Pitelka and Ashmun 1985). Numerous reports show PI increases the performance of ramets growing in stressful microenvironments (i.e. low nutrient, light or water availability) resulting from resources supplied by ramets growing in less stressful microenvironments (Hartnett and Bazzaz 1983, Saitoh et al. 2006, Campoy et al. 2017). It has therefore been hypothesized that PI is particularly important in habitats characterized by heterogenous resource availability or steep stress gradients (Stuefer et al. 1994, Shumway 1995, Pennings and Callaway 2000). This can even be reflected in intraspecific differences in the capacity to share nutrients and energy among ramets, as demonstrated by studies on genotypes of *Fragaria chiloensis* from habitats with different resource heterogeneity (Alpert et al. 2003, Roiloa et al. 2007). More recently, PI has received increasing attention in invasion biology, as many of the world's most invasive plants in both aquatic and terrestrial ecosystems are clonal (Song et al. 2013, Campoy et al. 2017).

At the interface of terrestrial and aquatic ecosystems, tidal marshes provide a wide array of critical ecosystem services (such as long-term carbon sequestration, pollutant retention and coastal protection) making them some of the economically most valuable ecosystems on Earth (Barbier et al. 2011, Kirwan and Megonigal 2013). However, tidal-marsh ecosystem services are threatened by a multitude of global-change factors, including the spread of invasive species (Kirwan and Megonigal 2013, Mueller et al. 2016, Davidson et al. 2018). Like other wetland ecosystems, tidal marshes are considered highly susceptible to plant invasions (Zedler and Kercher 2005) and invasions or range expansions of tall-growing clonal grasses are occurring at rapid rates in tidal marshes worldwide. Prominent examples are the invasions of *Phragmites australis* in North America (Saltonstall 2002), several species of the genus *Spartina* globally (Daehler and Strong 1996, Kriwoken and Hedge 2000, Nehring and Hesse 2008, Tong et al. 2012), and *Elymus athericus* in Europe (Bockelmann and Neuhaus 1999). Anthropogenic impacts arising from marine shipping trade, intentional planting for coastal engineering, and eutrophication are considered important triggers of these invasions at global and landscape scales (Vitousek et al. 1997, Sorte et al. 2013). However, the plant morphological and physiological basis for successful establishment and spread of the species at smaller scales (i.e. within ecosystems) is yet poorly understood (Mozdzer et al. 2016).

PI as a physiological trait could be a key mechanism for the expansion of clonal invasive plants in tidal marshes, because resource availability is highly heterogenous in these ecosystems (Schrama et al. 2013). That is, competition for nutrients decreases and nutrient inputs increase with decreasing surface elevation, i.e. increasing flooding frequency (Bertness and Callaway 1994, Mueller et al. 2020a). Expansion into lower elevated microhabitats could therefore alleviate plants from nutrient limitation. Indeed, a frequently observed invasion pattern in tidal marshes is the expansion from high-elevation and high-competition into low-elevation microhabitats, as for instance reported for the invasion of *Phragmites australis* and *Elymus athericus* (Mozdzer et al. 2016, Nolte et al. 2019). However, increasing flooding frequency also leads to oxygen-depleted soil conditions and high salinity in the root zone, two interacting stressors that can interfere with root development and plant nutrient uptake in tidal marshes (Alldred et al. 2017). Particularly seawater-derived sulfate supply and the resulting accumulation of phytotoxic sulfides under anoxic conditions are known to suppress nutrient assimilation by roots (Bradley and Morris 1990). Nutrient transfer from parent to offspring ramets via PI could therefore play a critical role for the process of clonal expansion into flooded soils, when offspring root development or nutrient uptake is impeded. However, the dynamics of nutrient transport between ramets in this process have not yet been studied.

The present study investigates PI in the salt marsh grass *Elymus athericus*. Although native to the European Atlantic coast between Portugal and Denmark, its fast range expansion within ecosystems represents one of the most prominent changes to the NW European salt-marsh landscape in recent decades (Bockelmann and Neuhaus 1999, Valéry et al. 2004, Veeneklaas et al. 2013). With a range originally restricted to rarely flooded high-marsh environments, *Elymus* has been invading into more frequently flooded low-marsh environment in the last decades. It forms large monospecific stands, which reduces small-scale plant species diversity and affects ecosystem services (Bockelmann and Neuhaus 1999, Valéry et al. 2004, Nolte et al. 2019). The progressing ecosystem-wide dominance of *Elymus* has been reported from several coastal regions, for example Mont Saint Michel Bay, France (Valéry et al. 2004), The Wash, UK (Norris et al. 1997) and the entire Wadden Sea region between the Netherlands and Denmark (Rupprecht et al. 2015, Esselink et al. 2017). Although several factors, including eutrophication and land-use change, have been discussed as important triggers for *Elymus*' invasion on the landscape-scale (Rupprecht et al. 2015, Nolte et al. 2019), the physiological mechanisms behind *Elymus*' high competitiveness at the small scale have not yet been investigated.

Another factor possibly contributing to *Elymus*' competitiveness could be its large degree of genotypic plasticity. Specifically, marked genetic differences between *Elymus* genotypes from high-marsh environments (in the following HM genotype) and genotypes from the low marsh (in the following LM genotype) are consistent across sites from the entire Wadden Sea region (Bockelmann et al. 2003). These genetic differences are also reflected in phenotypic characteristics: HM genotypes are grey-blue in color and produce tall shoots in dense stands, whereas LM genotypes are light green, produce more ramets and grow in a patchier distribution (Bockelmann et al. 2003). The physiological differences between *Elymus* genotypes, however, have not been assessed.

This also includes PI, a trait that is possibly tightly linked to *Elymus*' fast expansion within sites, and which has been demonstrated to strongly differ between genotypes in terrestrial plants (Lötscher and Hay 1997, Alpert et al. 2003, Roiloa et al. 2007).

Here we investigate the role of PI in the invasion process of *Elymus* from drained (representing high elevation) into flooded (representing low elevation) soil environments. In a factorial mesocosm experiment, PI was studied by applying a nitrogen stable-isotope approach to trace nutrient translocation between ramets. To test if genotypic differences interact with the species' capacity to physiologically integrate from drained into flooded soils, we included both HM and LM genotypes in our study. We hypothesized 1) that integrated offspring ramets show higher growth performance than ramets without connection to their parent. We further hypothesized 2) that integrated offspring ramets under flooded soil conditions are stressed and consequently produce less biomass and require more support (nutrient supply) from parent ramets than those in drained soils. Because the LM genotype is expected to be more adapted to flooding stress, we hypothesize 3) that LM connected offspring ramets produces more biomass under flooded conditions and require less support (nutrient supply) from parent ramets than HM connected offspring ramets.

Material and methods

Plant material

Plants were collected in April 2015 from stands on the Dutch island Schiermonnikoog that have previously been demonstrated to be dominated by genetically distinct populations of *Elymus*, i.e. HM and LM genotypes, which were further confirmed based on their phenotypic differences in leaf color and morphology (Bockelmann et al. 2003). Topsoils at the site are well-aerated under non-flooded conditions with redox potentials of 200–400 mV and salinity < 10 ppt (Howison et al. 2015). The plants were transferred into pots and kept in a common garden (i.e. all pots exposed to the identical environmental conditions) at Universität Hamburg for 12 months before the experiment commenced. Clonal plant growth led to the emergence of new individuals during this period, which were used for the experiment. New individuals of LM and HM genotypes were still phenotypically distinct after 12 months.

Experimental design and setup

The experiment included three factors in a full-factorial design, namely physiological integration (integrated versus severed rhizome), flooding (flooded versus drained offspring ramet) and genotype (LM versus HM genotype) (Fig. 1). Replication within each group was $n = 10$.

The experiment was conducted in 2016 for 84 days in the greenhouse of the Institute of Plant Science and Microbiology, Universität Hamburg, Germany. Forty parent ramets of each genotype that developed a rhizome of 8–10 cm length were selected. These ramets were planted separately in plastic pots ($10 \times 10 \times 12$ cm) filled with homogenized soil from a salt marsh at Dieksanderkoog (Schleswig-Holstein, Germany). Soils were collected from a high-elevation site within the marsh dominated by *Elymus* and characterized by similar topsoil salinity (< 10 ppt) and redox conditions (> 400 mV) as the site of plant collection (Mueller et al. 2017 and unpubl.). Each pot was connected to an identical pot via a silicone tube. Rhizomes were put through the silicone tube and into the soil
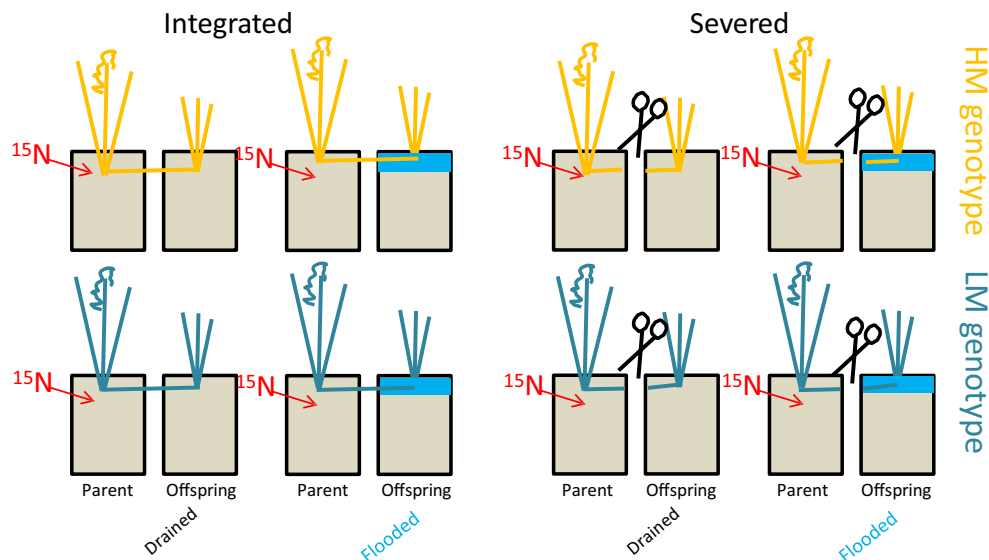

Figure 1. Schematic representation of the three-factorial experiment with the factor: integration (integrated versus severed), flooding (drained versus flooded) and genotype (low-marsh genotype versus high-marsh genotype). Parent ramets were ^{15}N labeled in the beginning of the experiment with 20 ml of 0.7 mM $^{15}NH_4^{15}NO_3$ (98 atom% ^{15}N).

433

of the other pot, where an offspring ramet established within 3–5 days. Pot positions were arranged randomly inside the greenhouse chamber and their positions were changed every week to avoid possible position effects due to e.g. heterogeneous light availability.

All parent plants were subjected to labeling with the heavy nitrogen isotope (^{15}N) by adding 20 ml of a 0.7 mM ammonium nitrate solution into the pot containing 98 atom% ^{15}N in form of $^{15}NH_4^{15}NO_3$. 20 ml unlabeled 0.7 mM NH_4NO_3 solution was added to the offspring pots.

To test for PI effects, half of the ramet pairs of each genotype were randomly selected and their rhizome was severed in the pot of the parent ramet, the other half was left intact (in the following referred to as integration treatment). The part of the rhizome sticking in the silicone tube remained intact. Both sides of the silicon tube were then filled with silicone sealant to prevent water transfer.

Half of the offspring ramets from each integration treatment and of each genotype were randomly selected for a flooding treatment. We used synthetic sea salt to create saltwater of 10‰ salinity, which was added to cover the soil surface (1 cm). Synthetic seawater was used to simulate a tidally induced flooding situation including sulfate supply to the soil system. To avoid salt build-up, freshwater was added to replace evaporation in the offspring pots throughout the experimental period. All other parent and offspring pots were watered with freshwater at regular intervals to maintain salinity and optimum moisture conditions.

Isotope analysis and data interpretation

Green leaf biomass and living root biomass were harvested, rinsed with deionized water, dried at 60°C until constant weight and homogenized. N- and C-isotope analysis was conducted using an isotope-ratio mass spectrometer linked to an element analyzer. Isotope calibration was conducted using the certified standard IAEA-NO-3 Potassium Nitrate (δ^{15}N = +4.7‰ air N_2, SD 0.2‰) and IAEA-600 Caffeine (δ^{13}C = −27.771‰ VPDB, SD 0.043‰). The isotopic compositions of all samples are reported using the standard δ-notation. We interpret δ^{15}N of offspring plant tissues to represent the relative importance of N transfer from mother ramets versus N acquisition via offspring root uptake. That is, higher δ^{15}N signatures represent a larger dependence of offspring ramets on N transfer. The δ^{13}C signature of plant tissue was used as a proxy for plant osmotic stress to test if flooding affected plant performance by controlling soil-water uptake via changes in salinity (Guy et al. 1980).

Statistical analysis

Three-way ANOVA was used to test for effects of integration (integrated versus severed rhizome; hypothesis 1), flooding (flooded versus drained offspring; hypothesis 2), and genotype (LM versus HM genotype; hypothesis 3) on above- and belowground biomass and plant-tissue δ^{13}C. Tukey's HSD tests were used for pair-wise comparisons. We tested for differences in

δ^{15}N between connected and severed offspring ramets using a non-parametric test (Wilcoxon's rank-sum test), because data were not normally distributed within groups. To test for the effects of flooding and genotype on δ^{15}N of integrated offspring ramets (hypotheses 2 and 3), two-way ANOVAs were conducted separately for above- and belowground biomass with flooding and genotype as main factors. Tukey's HSD tests were used for pair-wise comparisons. Finally, we explored relationships between N- and C-isotopic signatures and biomass parameters (aboveground biomass, belowground biomass, aboveground-to-belowground biomass ratio) using linear regression. Statistical analyses were carried out using the software package R ver. 3.5.0 (<www.r-project.org>).

Results

Biomass

Aboveground biomass of offspring ramets was significantly affected by the factors integration and genotype, whereas the effect of flooding on aboveground biomass was only marginally significant (Fig. 2, Table 1). Integration enhanced
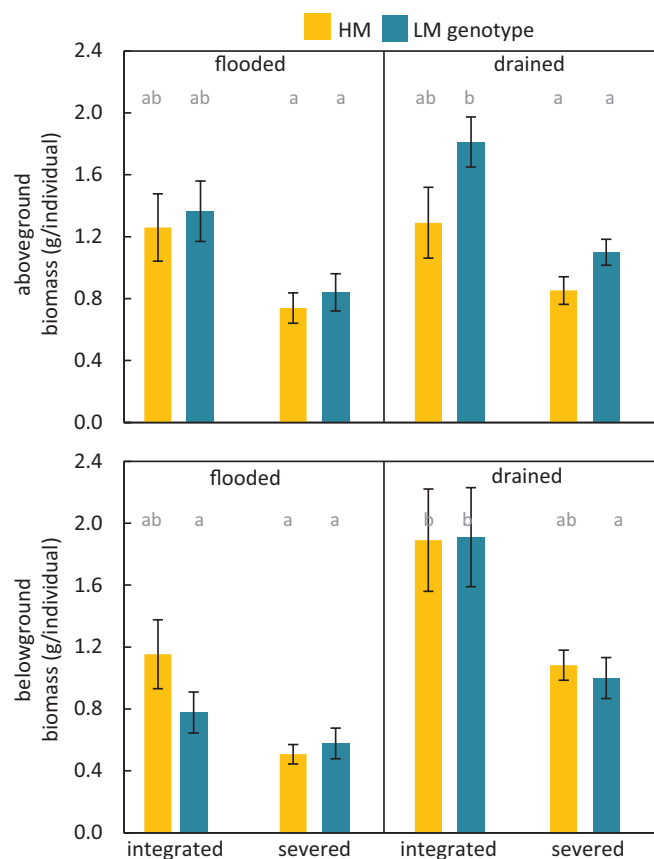

Figure 2. Aboveground (top panel) and belowground (bottom panel) biomass of offspring ramets at different factor combinations (flooding × integration × genotype). Shown are mean values ± SE; bars not connected by the same letter are significantly different at p ≤ 0.05 based on Tukey's HSD tests; n = 10.

Table 1. Results of three-way ANOVAs testing for main and interaction effects of the factors genotype, integration and flooding on aboveground and belowground biomass of *Elymus* offspring ramets after 84 days of growth. Values are highlighted in bold at p ≤ 0.1; n = 10.

Factor	df	Aboveground		Belowground	
		F	p	F	p
Genotype	1	**4.7**	**0.034**	0.4	0.514
Integration	1	**23.7**	**<0.001**	**20.6**	**<0.001**
Flooding	1	**3.6**	**0.064**	**25.8**	**<0.001**
Genotype × Integration	1	0.4	0.542	0.4	0.546
Genotype × Flooding	1	1.6	0.217	0.2	0.666
Integration × Flooding	1	0.1	0.815	2.4	0.126
Genotype × Integration × Flooding	1	0.4	0.549	0.9	0.335
Residuals	72				

aboveground biomass on average by 62% (Fig. 2). LM genotype ramets built 27% more aboveground biomass than the HM genotype ramets. No interaction effects among factors were found on aboveground biomass (Table 1).

Integration and flooding significantly affected belowground biomass, whereas genotype had no significant effect (Fig. 2, Table 1). Belowground biomass in integrated ramets was 81% higher than in severed ramets. Offspring ramets grown under drained conditions had 95% more belowground biomass than the ones grown under flooded conditions. There were no interaction effects among treatments on belowground biomass (Table 1, Fig. 2).

^{15}N translocation

δ^{15}N in integrated ramets (343.55‰) was significantly higher than in severed ramets (13.11‰), p < 0.0001. These data prove the underlying assumption that ^{15}N was translocated from parent ramets to offspring ramets via the rhizome,

and that the experimental set up prevented any undesirable ^{15}N translocation between mother and offspring mesocosm via other mechanisms. The following results reporting on ^{15}N translocation are therefore restricted to the data on integrated offspring ramets.

Aboveground biomass was more enriched in ^{15}N under flooded (522 ± 209‰) versus drained (369 ± 221‰) conditions (Fig. 3, Table 2). Similarly, belowground biomass was more ^{15}N-enriched under flooded conditions (411 ± 170‰ versus 290 ± 154‰). Genotype and the interaction of flooding and genotype did not affect δ^{15}N in aboveground and belowground biomass (Table 2); however, pair-wise comparisons demonstrated that the positive effect of flooding on δ^{15}N was more pronounced and only significant in the LM genotype (Fig. 3).

Both aboveground and belowground δ^{15}N were significantly and positively related to offspring above/belowground biomass ratios (r = 0.487 and 0.499; p = 0.002 and 0.001, respectively). Aboveground δ^{15}N was furthermore significantly and negatively related to offspring belowground biomass (r = −0.336; p = 0.03).

Discussion

Flooding increases the importance of physiological integration

Anthropogenic impacts arising from marine shipping trade, intentional planting for coastal engineering, and eutrophication are considered important triggers of wetland plant invasions at global and landscape scales (Vitousek et al. 1997, Sorte et al. 2013). Yet, the morphological and physiological basis for the successful establishment and subsequent spread of invasive wetland plants is poorly understood. Here we demonstrate the importance of PI for the growth of *Elymus* offspring ramets and thereby the species' capacity for fast vegetative spread. In accordance with our first hypothesis, PI drastically increased offspring biomass. Like other prominent
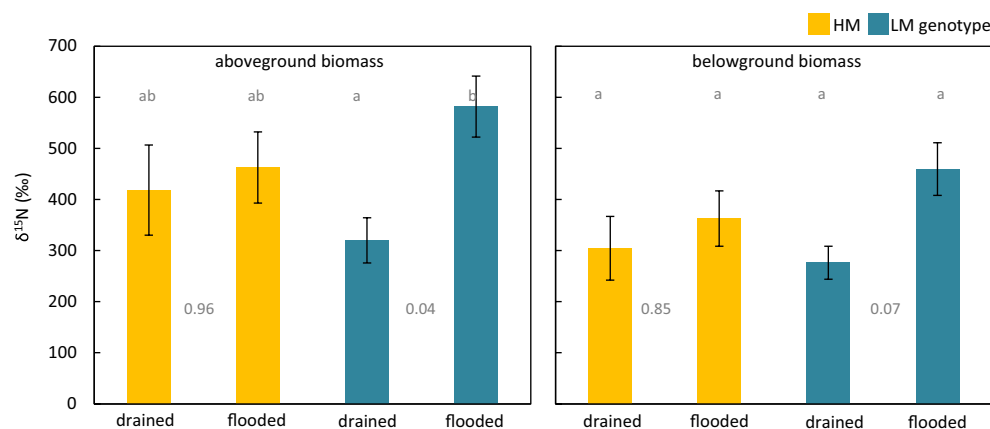

Figure 3. Above- and belowground biomass δ^{15}N of integrated offspring ramets of high-marsh (HM) and low-marsh (LM) genotypes under drained versus flooded conditions. Shown are mean values ± SE. Bars not connected by the same letter within the same panel are significantly different at p ≤ 0.05 based on Tukey's HSD tests, precise p-values are given between bars; n = 10.

Table 2. Results of two-way ANOVAs testing for main and interaction effects of the factors genotype, and flooding on $\delta^{15}N$ of aboveground and belowground biomass in integrated offspring ramets of *Elymus*. Values are highlighted in bold at $p \leq 0.05$; n =10.

Factor	df	Aboveground		Belowground	
		F	p	F	p
Genotype	1	0.0	0.878	0.45	0.509
Flooding	1	**5.2**	**0.029**	**5.5**	**0.024**
Genotype × Flooding	1	2.6	0.115	1.5	0.231
Residuals	76				

wetland invaders, *Elymus* spreads from rarely flooded and low-stress into frequently flooded and high-stress soil microenvironments. In accordance with our second hypothesis, our results demonstrate that the spread into flooded soils is associated with a marked reduction in biomass when compared to the spread into drained soils (Fig. 2).

Furthermore, our data show that offspring ramets in flooded soils are more dependent on nutrient supply from mother ramets than offspring ramets under drained conditions. That is, higher $\delta^{15}N$ values in belowground and aboveground biomass of flooded offspring ramets (Fig. 3) suggest a greater importance of N transfer from mother ramets versus N acquisition via offspring root uptake. In support of this notion, biomass reductions in response to flooding were clearly more pronounced below- than aboveground (Fig. 2). More importantly, both above- and belowground $\delta^{15}N$ were significantly and positively related to offspring above/belowground-biomass ratios, and aboveground $\delta^{15}N$ was significantly and negatively related to offspring belowground biomass. These relationships suggest an increased dependence on N transfer from parent ramets when offspring belowground biomass is insufficiently developed.

Our findings agree with studies on PI in terrestrial environments demonstrating reduced allocation to belowground biomass in offspring ramets under stressful and nutrient poor conditions, while aboveground biomass is maintained or reduced to a lower degree (Roiloa and Hutchings 2013, Lechuga-Lago et al. 2016). These studies proposed that PI-facilitated maintenance of aboveground biomass and the lower need to invest in belowground biomass under high-stress conditions can increase the spatial expansion pace of invasive plants in terrestrial environments. Our data provide support for the applicability of this concept to wetlands.

The objective of our experiment was to simulate a flooding situation with seawater. Thus, our experimental design was not meant to disentangle effects of salinity and anoxia. Anoxia, as a separate stressor, can impede plant nutrient uptake via energetic constraints of the root tissue as oxygen supply from aboveground tissues is insufficient to maintain aerobic dissimilation (Colmer and Flowers 2008). High soil salinity, as a separate stressor, can induce osmotic and ionic stresses in plants that interfere with nutrient uptake, although halophytes are equipped with several adaptations to reduce their negative impacts (Brown et al. 2006, Engels et al. 2011, González 2019). However, the most prominent stressor to

impede nutrient uptake in marsh plants is the accumulation of phytotoxic sulfides in the root zone (Bradley and Morris 1990, Alldred et al. 2017). As sulfide is generated by the reduction of seawater-derived sulfate ions, it represents a stressor that results from the concurrent incidence of salinity and anoxia. Thus, studies evaluating the separate effects of anoxia or salinity on plant performance are certainly insightful from a mechanistic perspective, but they are unlikely to capture the plant response to realistic flooding-induced stress levels (Veldhuis et al. 2019).

For the interpretation of our results it is nevertheless important to exclude that osmotic stress, which could have been strong in both the flooded and drained treatment, drove the observed plant biomass response to flooding. Specifically, greater belowground biomass in unflooded treatments could have resulted from higher osmotic stress in dry but saline soils (Chaves et al. 2002), so that relatively lower belowground biomass in the flooded treatment would falsely be interpreted as a stress response. However, aboveground $\delta^{13}C$ values, an established proxy for drought- and salinity-induced osmotic stress impacting stomatal conductance and water-use efficiency (Guy et al. 1980, Condon et al. 1987, Read et al. 1991), did not differ between flooded (29.5 ± 1.0‰) and drained (29.8 ± 0.9‰) treatments ($F_{1,72} = 1.8$, p > 0.1). This lack of a flooding effect on $\delta^{13}C$ was consistent within all integration and genotype treatments (data not shown), suggesting that osmotic stress effects were negligible in the present study.

Genotype effects

Genotypes of the same plant species can differ strongly in their capacity to invade new wetland habitats and outcompete other species (Saltonstall 2002). Yet, little is known about the intraspecific trait variability responsible for these differences (Mozdzer and Zieman 2010, Mozdzer et al. 2010). PI has been proposed as one key trait of invasive species, enabling their rapid spread within ecosystems (Song et al. 2013), but few studies have assessed its intraspecific variability in invasive plants (Campoy et al. 2017) and, to our knowledge, none in wetland invaders. We hypothesized that the LM genotype of *Elymus* is more adapted to frequent flooding and reducing soil conditions than the HM genotype, and consequently that offspring ramets of the LM genotype produce more biomass in the flooded treatment. We reject this hypothesis, because no significant interaction effect of genotype and flooding on biomass production was found (Table 1). Interestingly, however, *Elymus* genotypes showed significant differences in biomass parameters irrespective of flooding and integration treatments. LM-genotype plants were characterized by higher aboveground biomass and aboveground/belowground biomass ratios. This greater investment in aboveground versus belowground biomass might enable a more efficient oxygen supply to root tissues under reducing soil conditions and can thus represent an adaptation to frequent flooding (Voesenek et al. 1989, Naidoo and Naidoo 1992, Tanaka et al. 2007, Langley et al. 2013). Furthermore,

greater aboveground biomass in the LM genotype can represent a strategy to outcompete low-growing plant species via shading, as previously demonstrated for other invasive plants, such as *Lonicera maackii* and *Phragmites australis* (McKinney and Goodell 2010, Holdredge and Bertness 2011).

Conclusions

Our findings demonstrate an important role of PI for the growth of *Elymus* offspring ramets and thereby for the capacity of the invasive grass for fast vegetative spread. Furthermore, offspring ramets under flooded (versus drained) conditions developed less belowground biomass but depended more on nutrient supply from parent ramets to maintain aboveground biomass. The ecosystem-scale consequences of these findings are not easy to predict. However, they support the hypothesis that PI-facilitated maintenance of aboveground biomass, and a lower need to invest in belowground biomass, under high-stress conditions can increase the spatial expansion pace of invasive plants, as previously stated for invasions in terrestrial ecosystems. Lastly, our results show marked differences in productivity and biomass allocation patterns between genotypes, highlighting the need to explore intraspecific trait variability to understand invasion processes.

Speculations

Despite missing genotype × flooding-interaction effects, our data still provide evidence of distinct intraspecific flooding responses. Specifically, the positive effect of flooding on $\delta^{15}N$ was more pronounced and only significant in the LM genotype (Fig. 3), indicating a greater dependency on nutrient supply from parent ramets (versus offspring root N uptake) in LM versus HM genotypes under flooding. Correspondingly, the strong negative effect of flooding on belowground biomass in integrated ramets was only significant in the LM genotype (Fig. 2). We think that these data reflect genotypic differences in allocation strategies during clonal spread: the HM genotype shows no adaptive response when integrating flooded instead of drained microenvironments, whereas the LM genotype reduces root production and increasingly relies on N transfer from parent ramets. This lower investment in the costly maintenance of belowground tissues under hypoxic conditions and the allocation of energy resources to aboveground processes could facilitate a faster spread of the LM genotype into or across abiotically stressful habitats. We therefore argue that intraspecific variability in clonal strategies represents an important factor determining plant species competitiveness and invasion success that deserves more attention by future research in the fields of plant ecology and invasion biology.

Data availability statement

The data used in this work are available at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.sj3tx963m> (Mueller et al. 2020b).

Author contributions

Peter Mueller: Conceptualization (equal); Data curation (equal); Formal analysis (lead); Investigation (supporting); Methodology (equal); Supervision (supporting); Validation (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (lead). **Hai T. Do**: Data curation (equal); Formal analysis (supporting); Investigation (lead); Methodology (equal); Writing – original draft (supporting); Writing – review and editing (supporting). **Christian Smit**: Conceptualization (supporting); Methodology (supporting); Resources (supporting); Supervision (supporting); Writing – review and editing (supporting). **Christoph Reisdorff**: Conceptualization (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Resources (supporting); Supervision (supporting); Writing – review and editing (supporting). **Kai Jensen**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (lead); Methodology (supporting); Project administration (equal); Resources (lead); Supervision (equal); Validation (equal); Writing – review and editing (supporting). **Stefanie Nolte**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Supervision (lead); Writing – review and editing (supporting).

References

Alldred, M. et al. 2017. Impact of salinity and nutrients on salt marsh stability. – Ecosphere 8: e02010.
Alpert, P. et al. 2003. Differences in performance between genotypes of *Fragaria chiloensis* with different degrees of resource sharing. – J. Ecol. 91: 27–35.
Barbier, E. B. et al. 2011. The value of estuarine and coastal ecosystem services. – Ecol. Monogr. 81: 169–193.
Bertness, M. D. and Callaway, R. 1994. Positive interactions in communities. – Trends Ecol. Evol. 9: 191–193.
Bockelmann, A. C. and Neuhaus, R. 1999. Competitive exclusion of *Elymus athericus* from a high-stress habitat in a European salt marsh. – J. Ecol. 87: 503–513.
Bockelmann, A. C. et al. 2003. Habitat differentiation vs. isolation-by-distance: the genetic population structure of *Elymus athericus* in European salt marshes. – Mol. Ecol. 12: 505–515.
Bradley, P. M. and Morris, J. T. 1990. Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in *Spartina alterniflora*. – Ecology 71: 282–287.

Brown, C. E. et al. 2006. The effects of salinity and soil drying on nutrient uptake and growth of *Spartina alterniflora* in a simulated tidal system. – Environ. Exp. Bot. 58: 140–148.

Campoy, J. G. et al. 2017. Resource-sharing strategies in ecotypes of the invasive clonal plant *Carpobrotus edulis*: specialization for abundance or scarcity of resources. – J. Plant Ecol. 10: 681–691.

Chaves, M. M. et al. 2002. How plants cope with water stress in the field. Photosynthesis and growth. – Ann. Bot. 89: 907–916.

Colmer, T. D. and Flowers, T. J. 2008. Flooding tolerance in halophytes. – New Phytol. 179: 964–74.

Condon, A. G. et al. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat . – Crop Sci. 27: 996–1001.

Daehler, C. C. and Strong, D. R. 1996. Status, prediction and prevention of introduced cordgrass *Spartina* spp. invasions in Pacific estuaries, USA. – Biol. Conserv. 78: 51–58.

Davidson, I. C. et al. 2018. Differential effects of biological invasions on coastal blue carbon: a global review and meta-analysis. – Global Change Biol. 24: 5218–5230.

Engels, J. G. et al. 2011. Stress tolerance and biotic interactions determine plant zonation patterns in estuarine marshes during seedling emergence and early establishment. – J. Ecol. 99: 277–287.

Esselink, P. et al. 2017. Salt marshes. – In: Kloepper, S. (ed.), Wadden Sea quality status report 2017. Common Wadden Sea Secretariat, Wilhelmshaven, Germany.

González, M. B. 2019. Adaptation of halophytes to different habitats. – IntechOpen Access. <http://dx.doi.org/10.5772/intechopen.87056>. <www.intechopen.com/books/seed-dormancy-and-germination/adaptation-of-halophytes-to-different-habitats>.

Guy, R. D. et al. 1980. Shifts in carbon isotope composition of two C3 halophytes under natural and artificial conditions. – Oecologia 44: 241–247.

Hartnett, D. C. and Bazzaz, F. A. 1983. Physiological integration among intraclonal ramets in *Solidago canadensis*. – Ecology 64: 779–788.

Holdredge, C. and Bertness, M. D. 2011. Litter legacy increases the competitive advantage of invasive *Phragmites australis* in New England wetlands. – Biol. Invas. 13: 423–433.

Howison, R. A. et al. 2015. Large herbivores change the direction of interactions within plant communities along a salt marsh stress gradient. – J. Veg. Sci. 26: 1159–1170.

Kirwan, M. L. and Megonigal, J. P. 2013. Tidal wetland stability in the face of human impacts and sea-level rise. – Nature 504: 53–60.

Kriwoken, L. K. and Hedge, P. 2000. Exotic species and estuaries: managing *Spartina anglica* in Tasmania, Australia. – Ocean Coast. Manage. 43: 573–584.

Langley, J. A. et al. 2013. Tidal marsh plant responses to elevated CO_2, nitrogen fertilization and sea level rise. – Global Change Biol. 19: 1495–503.

Lechuga-Lago, Y. et al. 2016. Clonal integration facilitates the colonization of drought environments by plant invaders. – AoB Plants 8: plw023.

Lötscher, M. and Hay, M. J. M. 1997. Genotypic differences in physiological integration, morphological plasticity and utilization of phosphorus induced by variation in phosphate supply in trifolium repens. – J. Ecol. 85: 341.

McKinney, A. M. and Goodell, K. 2010. Shading by invasive shrub reduces seed production and pollinator services in a native herb. – Biol. Invas. 12: 2751–2763.

Mozdzer, T. J. and Zieman, J. C. 2010. Ecophysiological differences between genetic lineages facilitate the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. – J. Ecol. 98: 451–458.

Mozdzer, T. J. et al. 2010. Nitrogen uptake by native and invasive temperate coastal macrophytes: importance of dissolved organic nitrogen. – Estuar. Coasts 33: 784–797.

Mozdzer, T. J. et al. 2016. Deep rooting and global change facilitate spread of invasive grass. – Biol. Invas. 18: 2619–2631.

Mueller, P. et al. 2016. Complex invader-ecosystem interactions and seasonality mediate the impact of non-native *Phragmites* on CH_4 emissions. – Biol. Invas. 18: 2635–2647.

Mueller, P. et al. 2017. Top–down control of carbon sequestration: grazing affects microbial structure and function in salt marsh soils: grazing. – Ecol. Appl. 27: 1435–1450.

Mueller, P. et al. 2020a. Unrecognized controls on microbial functioning in blue carbon ecosystems: the role of mineral enzyme stabilization and allochthonous substrate supply. – Ecol. Evol. 10: 998–1011.

Mueller, P. et al. 2020b. Data from: With a little help from my friends: physiological integration facilitates invasion of wetland grass *Elymus athericus* into flooded soils. – Dryad Digital Repository, <http://dx.doi.org/10.5061/dryad.sj3tx963m>.

Naidoo, G. and Naidoo, S. 1992. Waterlogging responses of *Sporobolus virginicus* (L.) Kunth. – Oecologia 90: 445–450.

Nehring, S. and Hesse, K. J. 2008. Invasive alien plants in marine protected areas: the *Spartina anglica* affair in the European Wadden Sea. – Biol. Invas. 10: 937–950.

Nolte, S. et al. 2019. *Elymus athericus* encroachment in Wadden Sea salt marshes is driven by surface elevation change. – Appl. Veg. Sci. 22: 454–464.

Norris, K. et al. 1997. The density of redshank *Tringa totanus* breeding on the salt-marshes of the wash in relation to habitat and its grazing management. – J. Appl. Ecol. 34: 999–1013.

Pennings, S. C. and Callaway, R. M. 2000. The advantages of clonal integration under different ecological conditions: a community-wide test. – Ecology 81: 709–716.

Pitelka, L. F. and Ashmun, J. W. 1985. Physiology and integration of ramets in clonal plants. – In: Jackson, J. B. C. (ed.), Population biology and evolution of clonal organisms. Yale Univ Press.

Read, J. J. et al. 1991. Carbon isotope discrimination, gas exchange and water-use efficiency in crested wheatgrass clones. – Crop Sci. 31: 1203–1208.

Roiloa, S. R. and Hutchings, M. J. 2013. The effects of physiological integration on biomass partitioning in plant modules: an experimental study with the stoloniferous herb *Glechoma hederacea*. – Plant Ecol. 214: 521–530.

Roiloa, S. R. et al. 2007. Greater capacity for division of labour in clones of *Fragaria chiloensis* from patchier habitats. – J. Ecol. 95: 397–405.

Rupprecht, F. et al. 2015. Succession in salt marshes – large-scale and long-term patterns after abandonment of grazing and drainage. – Appl. Veg. Sci. 18: 86–98.

Saitoh, T. et al. 2006. Effects of resource heterogeneity on nitrogen translocation within clonal fragments of *Sasa palmata*: an isotopic (^{15}N) assessment. – Ann. Bot. 98: 657–663.

Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. – Proc. Natl Acad. Sci. USA 99: 2445–2449.

Schrama, M. et al. 2013. Food web assembly at the landscape scale: using stable isotopes to reveal changes in trophic structure during succession. – Ecosystems 16: 627–638.

Shumway, S. W. 1995. Physiological integration among clonal ramets during invasion of disturbance patches in a New England salt marsh. – Ann. Bot. 76: 225–233.

Song, Y. B. et al. 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. – Oecologia 171: 317–327.

Sorte, C. J. B. et al. 2013. Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. – Ecol. Lett. 16: 261–270.

Stuefer, J. F. et al. 1994. High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments. – J. Ecol. 82: 511–518.

Tanaka, N. et al. 2007. Effect of broken dead culms of *Phragmites australis* on radial oxygen loss in relation to radiation and temperature. – Hydrobiologia 583: 165–172.

Tong, C. et al. 2012. Invasive alien plants increase CH_4 emissions from a subtropical tidal estuarine wetland. – Biogeochemistry 111: 677–693.

Valéry, L. et al. 2004. Impact of the invasive native species *Elymus athericus* on carbon pools in a salt marsh. – Wetlands 24: 268–276.

Veeneklaas, R. M. et al. 2013. Spatio–temporal dynamics of the invasive plant species *Elytrigia atherica* on natural salt marshes. – Appl. Veg. Sci. 16: 205–216.

Veldhuis, E. R. et al. 2019. Plant stress-tolerance traits predict salt marsh vegetation patterning. – Front. Mar. Sci. 5: 501.

Vitousek, P. M. et al. 1997. Introduced species: a significant component of human-caused global change. – N. Z. J. Ecol. 21: 1–16.

Voesenek, L. A. C. J. et al. 1989. Root and shoot development of *Rumex* species under waterlogged conditions. – Can. J. Bot. 67: 1865–1869.

Zedler, J. B. and Kercher, S. 2005. Wetland resources: status, trends, ecosystem services and restorability. – Annu. Rev. Environ. Resour. 30: 39–74.