General plant strategies and functions in wetlands: global trait-based analyses

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Citation

Pan, Y. (2020, September 15). *General plant strategies and functions in wetlands: global trait-based analyses*. Retrieved from https://hdl.handle.net/1887/136753

Author: Pan, Y.
Title: General plant strategies and functions in wetlands: global trait-based analyses
Issue Date: 2020-09-15

Chapter 4

Are ecophysiological adaptive traits decoupled from leaf economics traits in wetlands?

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Functional Ecology, **2019, 33: 1202–1210.**

doi.org/10.1111/1365-2435.13329

Abstract

- Wetland plants have developed a suite of traits, such as aerenchyma, radial oxygen loss, and leaf gas films, to adapt to the wetland environment characterised by e.g. a low redox potential and a lack of electron acceptors. These ecophysiological traits are critical for the survival and physiological functioning of wetland plants. Most studies on these traits typically focus on a single trait and a single or few species at the time.
- Next to these traits, traits of the leaf economics spectrum (LES) that reflect resources acquisition and allocation in plant species have also been frequently measured in wetlands. However, the performance of the LES has rarely been examined among wetland plants.
- Both suites of traits are critical for -but affect different aspects of- wetland plant functioning and survival. The interactions between them, potentially causing synergies or trade-offs, reflect wetland plant strategies to simultaneously deal with stress tolerance and resources utilization, and have ramifications for the functioning of wetland ecosystems.
- Based on a literature review and quantitative analysis of available data, we provide evidence suggesting that LES and ecophysiological traits may be decoupled (e.g., for root porosity & radial oxygen loss vs. leaf nitrogen) or coupled (e.g., for iron tolerance vs. SLA) in wetlands, depending on the trait combination concerned. This rather complex relationship between wetland adaptive traits and LES traits indicates that there can be multiple mechanisms behind the strategies of wetland plants.
- We further illustrate how adaptive and LES traits together contribute to wetland ecosystem functions, such as denitrification and methane emission. We highlight that both suites of traits should be considered simultaneously when applying trait-based approaches to wetland ecology.

4.1 Introduction

Wetland ecosystems include a wide variety of fresh and saltwater habitats (including for example marshes, peatlands, mangroves, rivers, lakes, intertidal mudflats and rice paddies) that are distinguished from terrestrial habitats by a different hydrological regime (Ramsar Convention Secretariat, 2013). This causes wetland ecosystems to have unique features in terms of oxygen availability, nutrient cycles, soil pH and redox potential. These deviating environmental conditions strongly affect the survival and functioning of wetland plants. In response, wetland plants have developed a suite of adaptive traits, including tolerance and escape traits, to waterlogging or inundation and other conditions characteristic of wetlands (Jackson & Armstrong, 1999; DeLaune & Pezeshki, 2001; Pezeshki & DeLaune, 2012). These traits are strongly related to wetland plant performance, sometimes even vital to their survival. Previous studies on these adaptive traits have commonly focused only on one or a few species at the individual level, which makes these adaptive traits hard to incorporate into trait-based wetland ecology. In contrast, leaf economics spectrum traits (LES) such as leaf nitrogen (leaf N), leaf phosphorus (leaf P), specific leaf area (SLA) and photosynthetic rate (A_{mass} or A_{area}) have received more attention, but do not include those traits that are considered vital to the survival of plants under wetland conditions in ecophysiological studies (Visser *et al.*, 2000b; van Bodegom *et al.*, 2005; Voesenek & Bailey-Serres, 2015).

Moreover, the functional importance of most traits is context-specific (Wright & Sutton-Grier, 2012; Baastrup-Spohr *et al.*, 2015; Shipley *et al.*, 2016). This context may well differ for wetland ecosystems compared to terrestrial ecosystems, because trait selection is strongly driven by environmental factors (DeLaune & Pezeshki, 2001; van Bodegom *et al.*, 2012). A recent review paper (Moor *et al.*, 2017) carefully reviewed both wetland adaptive traits and LES traits as well as their effect on ecosystem functioning, and the authors suggested not to simply employ the LES/PES (Plant Economics Spectrum) to understand wetland ecosystems, since they vary widely in site conditions (bogs, peatland, marsh etc.). The study called for the inclusion of LES/PES and adaptive traits to get a better understanding of wetland ecology. To move towards this goal we need to understand how these two groups of traits, if taken as the two major trait axes, position in relation to each other. In other words, it is important to disentangle the different roles that wetland adaptive traits and LES traits play in plant survival and resource utilization respectively, their relationships being orthogonal (reflecting a decoupling) or coordinated (reflecting coupling through synergies or trade-offs), and the consequent effects on ecosystem functioning.

The adaptive response and the physiological mechanisms of adaptive strategies to wetland conditions have been carefully examined in ecophysiological studies, which have shown adaptation in traits in relation to root morphology and plant physiology (Laan *et al.*, 1989; Colmer, 2003a; van Bodegom *et al.*, 2005). For instance, plants can adapt to cope with the oxygen deficiency associated to waterlogging/flooding by developing adventitious roots or aerenchyma in shoots or roots (Justin & Armstrong, 1987; Blom *et al.*, 1994; Wright *et al.*, 2017a), or enhancing root porosity (Justin & Armstrong, 1987; Garthwaite *et al.*, 2003). Likewise, radial oxygen loss (ROL) protects plant roots from anaerobic stress (Lemoine *et al.*, 2012), whereas barriers to ROL in basal zones enhance longitudinal oxygen diffusion towards the apex (Colmer, 2003a). Phytohormones such as ethylene, gibberellin and abscisic acid also play important roles in changing cellular and organ structure that alleviate the oxygen deficiency (Vartapetian & Jackson, 1997; Bailey-Serres & Voesenek, 2008). Most of these primarily ecophysiological studies on wetland plants, though, are limited to an experiment-based assessment of one individual trait for a few species at a time. Unfortunately, it is rather difficult to scale up results from such detailed studies to the impacts of different plants and communities on wetland ecosystem functioning. Therefore, we need to integrate these ecophysiological traits into a more general ecological framework (Fig. 4.1a).

There is some circumstantial evidence that wetland adaptive traits may be orthogonal to (i.e. independent of or decoupled from) LES/PES: wetland adaptive traits are the premise of plant existence in wetlands since they are vital to the survival of plants under hazardous anaerobic conditions. Based on that premise, one may expect trait selection processes in wetlands to be strong. At the same time, while LES traits are principally constrained by nutrient availability (e.g. Maire et al. 2015), wetland habitats span a wide fertility gradient from very infertile bogs to very fertile floodplains/marshes at a global scale. This provides the conditions to allow for a full range of leaf N if wetland adaptive traits are orthogonal to LES/PES (Fig. 4.1b). However, if trade-offs between the two axes predominate, one would expect only a subset of LES/PES would remain available for wetlands (Fig. 4.1c). The wide variety of growth strategies in wetlands, from conservative strategies associated with e.g. bogs to acquisitive strategies in highly productive systems such as reed lands, suggests that wetland plants can sufficiently develop adaptive traits to cope with multiple and varied wetland conditions. This pattern also suggests an orthogonal relationship between adaptive traits and LES/PES traits.

In this paper, we present an exploratory analysis to quantify the relationships between wetland adaptive traits and LES/PES traits. We hypothesize that adaptive traits are principally decoupled from LES/PES traits in wetlands, assuming that these adaptive traits are not costly to have. Consequently, we predict that we will see a wide range of LES/PES in wetland plants. Using published and unpublished data, we assess the relationship between wetland adaptive traits and LES/PES traits. Then we illustrate how wetland adaptive traits and LES/PES traits together impact wetland ecosystem functioning.

While the lack of integration of wetland adaptive traits into more generic trait-based approaches has formed a barrier to the direct employment of trait-based approaches to wetland ecosystems to date, we propose that a more comprehensive understanding of wetland ecology can be obtained through the quantification of the relationships between the two suites of traits. This will also allow us to make better-informed decisions with respect to one of the standard dilemmas in trait-based community ecology: the choice of measuring traits for ease of measurements and low cost vs. functional/mechanistic importance (Lavorel & Garnier, 2002; Wright *et al.*, 2010).

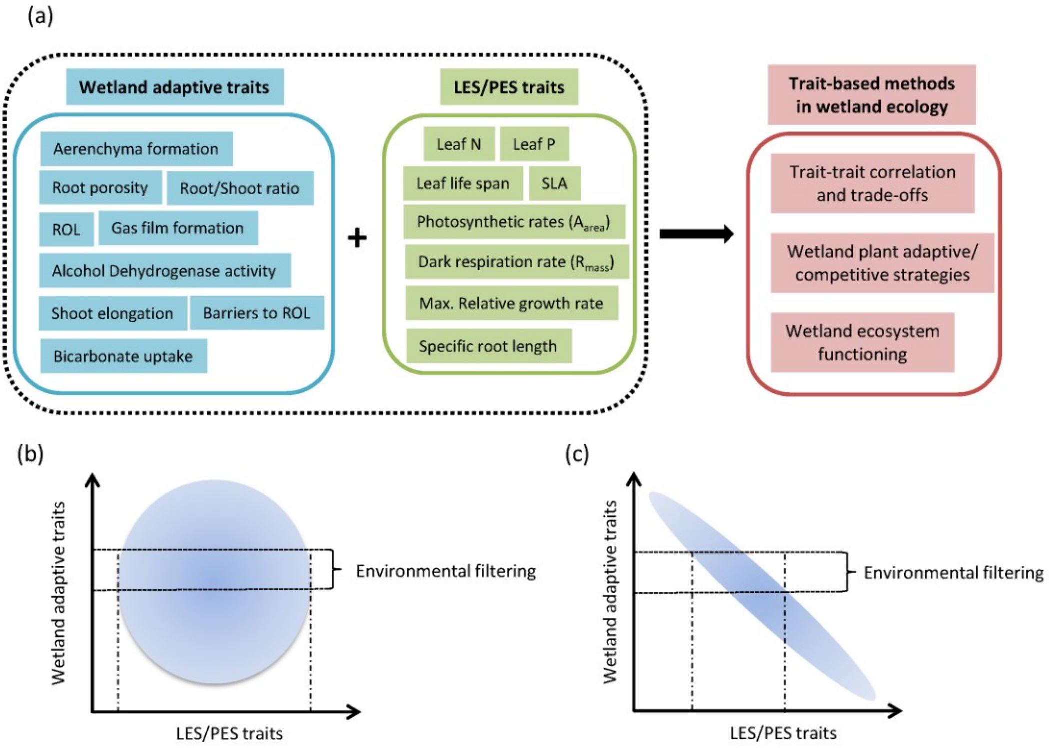

Figure 4.1 A summary of most commonly studied wetland adaptive traits and LES/PES traits (a); the relationships between these two suites of traits determine wetland plant adaptive and competitive strategies, and wetland ecological functioning. If wetland adaptive traits are orthogonal to LES/PES, even if environmental filtering to a specific setting of the water regime selects a subset of adaptive traits, almost a full range of LES/PES trait values would still be visible amongst wetland species (b). If trade-offs are predominant, environmental filtering of wetland conditions selects a subset of adaptive traits, consequently only a corresponding subset of LES/PES remains (c).

4.2 Literature review on the relationships between wetland adaptive traits and LES/PES

Some trade-offs among wetland adaptive traits and nutrient uptake have been described. In general, wetland plants may experience more nutrient stress than other plants under similar conditions of nutrient availability, because some adaptations to oxygen or redox stress result in a reduced adaptation to nutrient stress (Silvertown *et al.*, 2015). In turn, this is likely to negatively affect leaf nutrient contents, which are part of LES/PES. For instance, decreasing root respiration and increasing aerenchyma leave less energy and active root biomass, respectively, for the active uptake of nutrients (van der Werf *et al.*, 1988). A root barrier that retards oxygen leakage may also reduce the efficiency of nutrient uptake (Colmer, 2003b), although studies suggest that symplastic aquaporin activity can prevent this effect (Rubinigg *et al.*, 2002). In some cases, cortical aerenchyma also inhibits nutrient transport (Hu *et al.*, 2014). Another trade-off includes a decrease in phosphate availability in the presence of ROL by the oxidation of Fe^{2+} in the rhizosphere, inducing the precipitation of phosphate with iron. If these trade-offs are representative of the strategies of wetland plant species, then wetland plants species should occupy the lower ranges of the LES/PES.

In the case of specific leaf area (SLA), such a relationship is rather complex as SLA may be seen as part of LES/PES and other plant strategy axes, such as the size axis (Wright *et al.*, 2010), and it may also relate to wetland plant's adaptation to water stress. For example, community mean SLA increased with flooding, suggesting that SLA contributed to the plant's waterlogging tolerance (Violle *et al.*, 2011). Also, Mommer et al. (2007) found, across nine species, that the internal oxygen partial pressure, the trait that enhances waterlogging tolerance in plants, was positively correlated to SLA and negatively correlated to leaf thickness and cuticle thickness (while plasticity in these traits was not). Another extensive meta-analysis, comparing tens of species, suggested that the link between tolerance to oxygen stress and SLA response was significant but rather weak (Douma *et al.*, 2012).

While the examples above suggest some coordination for individual trait sets, when analysing tolerance towards waterlogging (presumably related to wetland adaptive traits) vs shade or drought (as related to LES/PES traits), a decoupling seems to prevail. A study of 806 shrubs/trees across continents suggested that correlations among shade, drought and waterlogging tolerance indices were significant but very weak (Niinemets & Valladares, 2006; Hallik *et al.*, 2009). This suggests that oxygen stress-related traits (waterlogging tolerance) might be decoupled from leaf economics traits (shade tolerance). Also, the fact that environmental drivers of the LES/PES traits are different from those driving wetland adaptive traits, suggests that some orthogonality may occur among these sets of traits.

Given the partially contradictory evidence listed in our qualitative literature review and since none of the above studies specifically tested the relationships of different trait axes, we provide an exploratory quantitative analysis in the next section.

4.3 Exploration of the relationships between wetland adaptive traits and LES/PES

To quantitatively explore the so far rather anecdotal and possibly contradictory relationships between wetland adaptive traits and LES/PES traits, we analysed a number of non-exhaustive published wetland ecophysiological studies and unpublished data sources, which presented trait measurements of both adaptive and LES/PES traits at the individual and species level under field or experimental conditions (see Appendix 4A for data description details). In our analysis, we assume that individual wetland plants exert their adaptive strategies in response to environmental stress, independent of whether the exposure happened in the field or at experimental conditions. For our exploratory analysis on the relationships between adaptive traits and LES/PES traits, we focused on three pairs of relationships (root porosity vs. leaf N, ROL vs. leaf N, iron tolerance vs. SLA), for which sufficient data were available for quantitative analysis. Root porosity and ROL are two very important ecophysiological adaptive traits at flooded conditions (Visser *et al.*, 2000b; Colmer, 2003b; Voesenek & Bailey-Serres, 2015), and reduced iron along with other reduced toxins is considered as the cause of the absence of non-wetland plants in wetland conditions (Snowden & Wheeler, 1993). Leaf N and SLA are leading traits driving the LES/PES axis (Wright *et al.*, 2004; Diaz *et al.*, 2016).

Previous studies have commonly observed a high degree of both interspecific and intraspecific variation in root porosity in wetland plants in response to oxygen stress (Lemoine *et al.*, 2012; Mei *et al.*, 2014), while leaf N varies according to soil fertility (following a gradient of acquisitive to conservative strategies) at the interspecific level (Ordoñez *et al.*, 2009; Maire *et al.*, 2015). To test the relationships between root porosity and leaf N, we collated data from three sources where both variables were measured on the same individuals (see Appendix 4A for further details): a) greenhouse experiment in which six wetland plant species were measured in a 2*2 factorial design with soil oxygen demand (SOD) and partial submergence as the main factor (van Bodegom *et al.*, 2008); b) a field study in Ukraine, where root porosity and leaf N of 53 species from forested/shrub wetlands and marsh habitats were measured at field conditions (unpublished data, Appendix 4A); c) a field study in the Netherlands, where root porosity and leaf N of 22 species from fens were measured at field conditions (unpublished data, Appendix 4A).

A linear regression between leaf N and (log-transformed) root porosity (Fig. 4.2) showed that, despite a significant correlation (*P*<0.01), the very low R^2 (adjusted R^2=0.030; n=267) indicates that only three per cent of the variation can be explained by the model. At a high sample size -such as here- a significant relationship does not necessarily imply ecological relevance (Yoccoz, 1991; Møller & Jennions, 2002). The low effect size effectively represents a decoupling (Fig. 4.2).

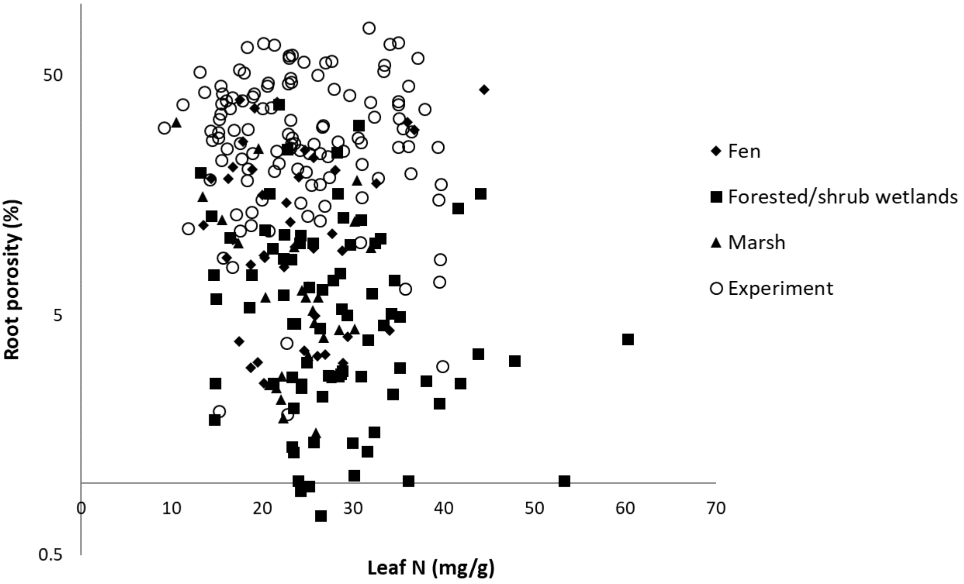

Figure 4.2 The relationships between root porosity and leaf N. The data are from measurements from a greenhouse experiment (van Bodegom *et al.*, 2008) and field measurements of three habitats: fen, marsh and forested/shrub wetlands (van Bodegom, unpublished data) (see details in Appendix 4A).

To test the relationships between ROL and leaf N, data were available from a greenhouse experiment, where five typical wet dune slack species were grown under all possible combinations of treatments with two (strongly differing) levels of light, fertility, reduced metal concentration, and water regime gradients (van Bodegom *et al.*, 2005). To be able to test this relationship, and because ROL data were heavily zero-inflated (92 out of 209 measurements showed no ROL), we grouped the ROL data into four classes in order to meet the normality assumption. The first class contained all 92 ROL observations, and the remaining 117 points were evenly divided into the other three classes in the ascending order (39 measurements for each bin). Subsequently, a linear model was run to test whether log-transformed leaf N varied as a function of ROL class. Despite a significant *P* value (which, again, we would consider induced by the large sample size), the overall lack of relationship (R^2=0.053) between log-transformed leaf N and ROL classes again suggested decoupling (Fig. 4.3).

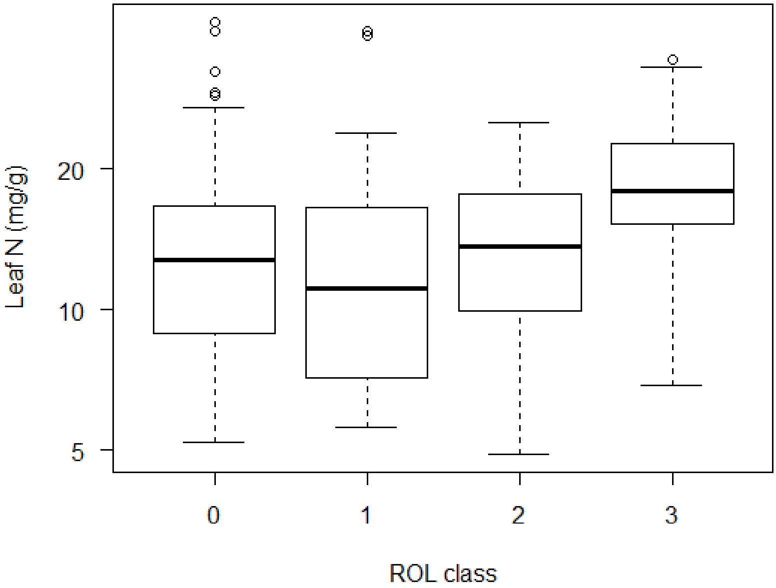

Figure 4.3 Box plot of leaf N across ROL class (adjusted R^2=0.053, $P<0.01$, n=209). Class 0: ROL=0 µmol O_2 h^{-1} per g root dry weight, n=92; class 1: ROL= 2.5 - 21.5 µmol O_2 h^{-1} per g root dry weight, n=39; class 2: ROL=21.6 - 85 µmol O_2 h^{-1} per g root dry weight, n=39; class 3: ROL=90 - 1212 µmol O_2 h^{-1} per g root dry weight, n=39. Data source: van Bodegom et al. (2005).

Results from these datasets suggest that: (i) potentially decoupled relationships between wetland adaptive traits and LES/PES traits may exist. Such decoupling indicates that the cost of, for example, root porosity formation might be relatively low for wetland plants and that a higher transportation capacity of oxygen to the rhizosphere (ROL) does not necessarily impede the nitrogen uptake capacity or the nitrogen utilization within plants, and (ii) almost a full range of leaf N was covered (3.4 to 60.3 mg/g) compared to the leaf N range of terrestrial plants worldwide (2.48 to 68.98 mg/g) (Diaz *et al.*, 2016). This full range of leaf N in wetland plants suggests that adaptation to wetland conditions are not necessarily costly (in agreement with Fig. 4.1b). This is also supported by evolutionary evidence: aquatic species have evolved at least 200 times from terrestrial species (Cook, 1999).

Another type of adaptive traits relates to the tolerance, rather than avoidance or escape, of stressful conditions in wetlands. As a key stress tolerance characteristic of wetland plants, iron tolerance has been long considered as the cause for differential survival, growth and distribution among wetland plants (Snowden & Wheeler, 1993). Iron reduction along with manganese reduction takes place in the redox sequence after the depletion of nitrate, and produces phytotoxic ferrous iron. The physiological mechanisms behind iron tolerance are probably a combination of oxidation of the rhizosphere (partly contributed by ROL) and a true tolerance for Fe^{2+}. Due to a lack of quantitative traits expressing these true iron tolerance mechanisms, we used the iron tolerance index proposed by Snowden & Wheeler 1993 as a proxy trait. In that study, an iron tolerance experiment was set up for 44 British fen species seedlings, cultivated under in 10% Rorison solution containing reduced iron (as ferrous sulphate). The iron tolerance index was estimated based on the impact of iron on the relative growth rate (RGR) in comparison with the RGR in a control group (Snowden & Wheeler, 1993). To test how iron tolerance relates to LES/PES traits, we derived SLA of the corresponding species (with the exception of *Oryza sativa* which was not available) from the LEDA database (Kleyer *et al.*, 2008). A linear regression between the iron tolerance index and SLA showed that the iron tolerance index decreased strongly and significantly with an increasing SLA (R^2=0.237, Fig. 4.4).

This pattern may indicate a true trade-off between iron tolerance trait and LES/PES traits. We hypothesize that tolerance -in contrast to avoidance or escape traits- may be costly and hence induce coupling with LES traits. It will require further experimental work to test this hypothesis more fully with other traits and in other systems. Such experimental evaluating should consider other LES traits than SLA in relation to tolerance, given that SLA may also directly play a role in wetland adaptation (as discussed in section 2).

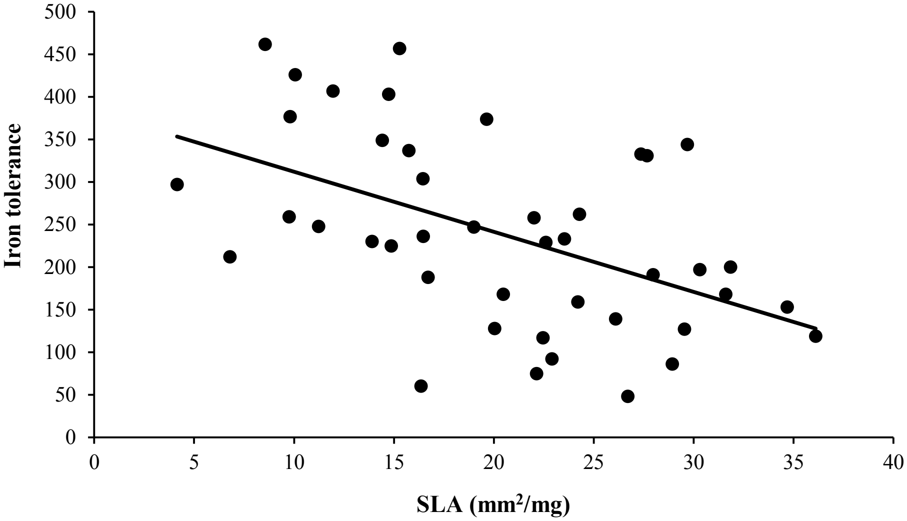

Figure 4.4 The relationship between SLA and iron tolerance (linear regression, adjusted R^2=0.237, P<0.001, n=43). SLA data were from the LEDA database (Kleyer *et al.*, 2008), iron tolerance data were estimated by Snowden and Wheeler (1993).

The three exploratory investigations presented here, suggest that both potentially coupled and decoupled relationships exist between wetland adaptive traits and LES/PES traits. The varied wetland adaptive traits may therefore not position along one trait axis, but some of them may be decoupled from one another. This implies that the selective forces in wetlands act in varied directions. The cost of developing a wetland adaption trait may vary, depending on the trait and the conditions. The varied relationships between the two suites of traits suggest a variety of possible adaptive strategies to deal with specific combinations of wetland conditions, including both flooding stress and nutrient acquisition aspects.

4.4 Scaling from wetland plant traits to ecosystem functioning

Considering the importance of wetland ecosystems to humans, with regards to ecosystem services including water quantity and quality regulation and habitat provisioning for water birds and fish (Zedler, 2003; Doherty *et al.*, 2014), more and more attention is being paid to understanding wetland ecosystem functioning. Trait-based approaches have been applied to characterize plant strategies and their effects on ecosystem functioning of wetlands (Moor *et al.*, 2017), but such studies have mainly focused on LES/PES traits (Douma *et al.*, 2012).

However, given the unique adaptive traits in wetland ecosystems, these need to be additionally considered to fully understand trait-based impacts on wetland ecosystem functioning. For instance, two important biogeochemical processes in wetlands, denitrification and methane production, depend on soil organic matter content - which are strongly influenced by community mean leaf nitrogen and carbon concentrations (LES/PES traits) (Koschorreck & Darwich, 2003) - and suitable aerobic/anaerobic conditions, which relate to ROL and root porosity (adaptive traits) (Engelhardt, 2006; Sutton-Grier *et al.*, 2013; Alldred & Baines, 2016).

Knowledge of the combined effects of adaptive traits and LES/PES traits can thus improve our understanding of denitrification and methane production, which is important for the sustainable management of wetlands, including the reduction of greenhouse gas emissions by wetlands and the relief of eutrophication in wetlands.

In addition to affecting the functioning of wetlands, wetland adaptive traits may also affect the community structure of wetlands in a complicated way. ROL relates to oxygen leaking from roots into the soil which results in microaerophilic conditions in the rhizosphere (e.g. van Bodegom and Scholten 2001). This allows detoxification of several potentially toxic compounds like S^{2-} and Fe^{2+}. The micro-aerophilic conditions induced by ROL do not only favour growth of the plant species that have ROL, but also facilitate the growth of less-adapted species that would not survive under purely anoxic soil conditions (Schat, 1984). As a consequence, the facilitation of these less-adapted species leads to a competition with the adapted species and a higher turnover of species than would have occurred otherwise (Grootjans *et al.*, 1998).

ROL also contributes to community composition in a more direct way, through its coupling of the nitrification and denitrification processes. Compared to cases in which ROL is absent, the increased availability of soil oxygen in communities with ROL induces nitrification. The produced nitrate diffuses into the anoxic bulk soil and is denitrified, and hence leads to increased nitrogen losses and decreased nutrient availability in wetland ecosystems (Reddy *et al.*, 1989; Adema *et al.*, 2005). Low nutrient availability makes it harder for competitors to invade, as many grow less effectively in such an environment. As a consequence, the community of stress-tolerating plant species, that grow less quickly at high nutrient levels, may remain more stable (Adema & Grootjans, 2003).

This feedback loop between ROL and denitrification is further complicated because both ROL (through oxygen supply for nitrification) and LES/PES traits by a combination of direct and indirect relations determine nitrate availability. If ROL is orthogonal to leaf N (as suggested by the exploratory analyses described above), these two influences on nitrate availability and hence denitrification may occur independently from each other. However, if there is a trade-off between ROL and leaf N, then nitrate sources can be limited by low ROL leading to a natural reduction of denitrification. In that case, a strong nitrification/denitrification coupling is not expected to occur. This example again demonstrates the importance of understanding the relationships between wetland adaptive traits and LES/PES.

Methane emission is another example that shows how wetland adaptive traits and LES/PES traits together affect ecosystem functioning. Methane production only takes place after most other alternative electron acceptors have been depleted. Both production and emission of methane are affected by wetland plants in many aspects. First of all, organic compounds released by root exudation can be used as electron donors for methane production (e.g. Aulakh et al. 2001). Secondly, oxygen released from the roots may be used by bacteria to oxidize methane to CO_2, decreasing methane emissions (e.g. van Bodegom et al. 2001). Thirdly, the aerenchyma channels of wetland plant species act as chimneys that effectively transport methane from the soil to the atmosphere. This plant-mediated transport pathway is much more effective than diffusion through the soil alone (e.g. van Bodegom et al. 2001b, Fig. 4.5) and decreases the probability of methane oxidation. The combination of adaptive traits and local conditions (such as temperature, water level, soil texture) determines whether the net effect of wetland plants is an amplification or decrease of methane emissions.

To further advance our quantitative understanding of strategies and functioning (including denitrification and methane emission) in wetlands, we identified a number of critical research topics that would benefit from an inclusive approach. First, we need to target specific pairs of wetland adaptive traits and LES/PES traits and study them quantitatively to better understand the nature and patterns of this relationship. Specifically, such analysis may test the hypothesis that tolerance traits may be coupled while avoidance and escape traits are not. Second, the drivers determining the selection of these different trait sets will have to be analysed. Third, based on an understanding of which traits do and which don’t couple to LES traits and under which conditions, combined with knowledge on how the interplay of adaptive and LES traits affect important wetland ecosystem functions, the variation in these ecosystem functions at the global scale can be quantified and understood. Such insights will help recognise the importance of wetland ecology in times of global change.

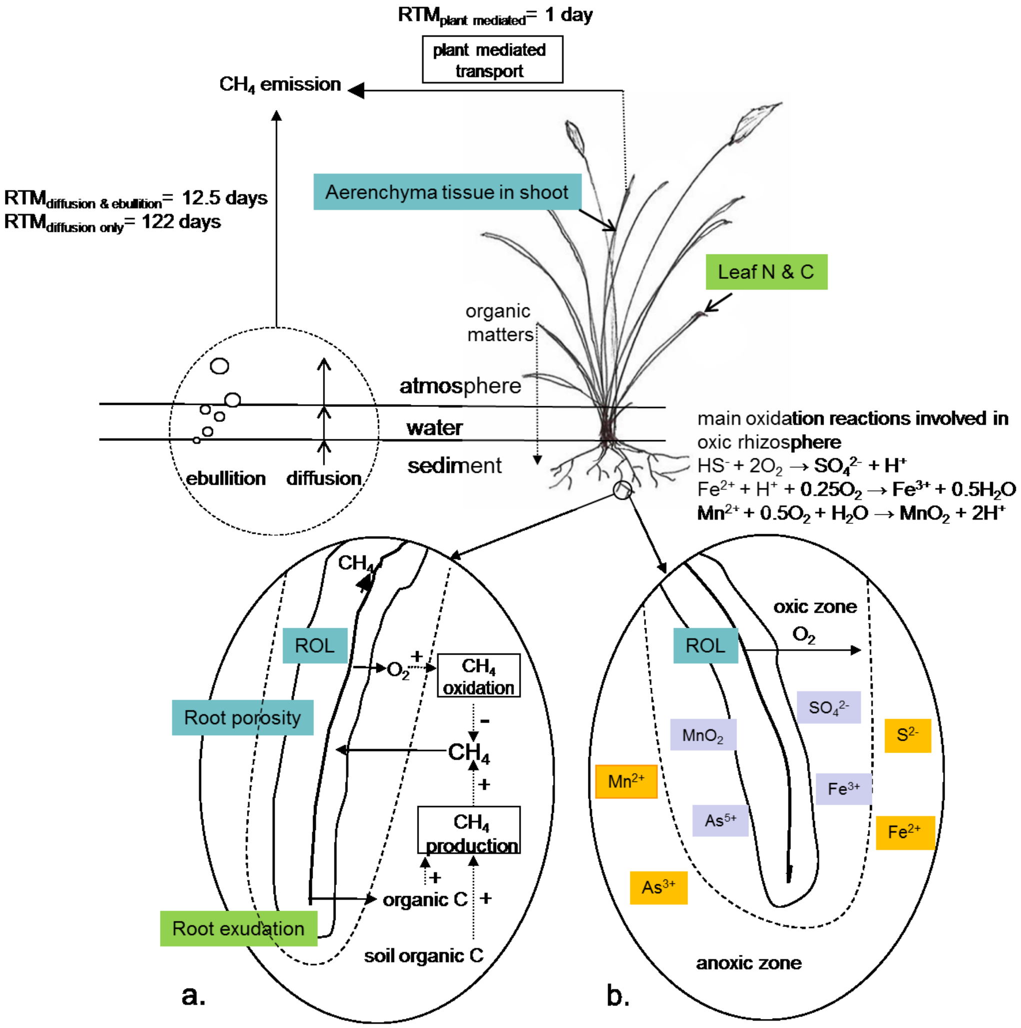

Figure 4.5 Schematic presentation of the wetland adaptive traits (in blue boxes) and LES/PES traits (in green boxes) impact on a.) the gas transportation through wetland plants and organic compounds release b.) oxidation reactions in oxic rhizosphere (with oxidized elements in purple boxes and reduced elements in orange boxes). The residence time of methane in soil (RTM) is based on data discussed in van Bodegom et al. (2001c).

4.5 Conclusions

By bridging the fields of study of wetland adaptive traits and LES/PES traits and their relationships, we can unravel wetland plant strategies and obtain a broader picture of wetland ecology. Our work provides a first exploration of such relationships through a qualitative literature review and a quantitative assessment between examples of the two suites of traits; this can be further explored in future wetland ecology research. Our analyses suggest both coupled and decoupled patterns do occur between wetland adaptive traits and LES/PES, and provides a first glimpse at the complex character of adaptation in wetland ecosystems. Further unravelling the relationships between the two suites of traits will be critical to understanding wetland ecosystem functioning, especially for those processes to which multiple traits contribute, such as denitrification and methane emissions, and that are globally important processes of greenhouse gas emissions. To fully reveal the patterns between adaptive traits and LES/PES traits, we are in need of global compilation and analysis of traits datasets.

4.6 Authors' contributions

P.M.v.B. conceived the study; Y.P., E.C. and P.M.v.B. developed the ideas; P.M.v.B. and Y.P. collected the data; Y.P. wrote the first draft and conducted the analyses. All authors contributed critically to the drafts and gave final approval for publication.

4.7 Acknowledgements

Y.P. is grateful to support from the China Scholarship Council (Grant No. 201606140037).

4.8 Data accessibility statement

Data deposited in the Dryad repository: http://doi.org/10.5061/dryad.4v1s6b5 Pan, Cieraad & Van Bodegom 2019

4.9 Supporting information

Appendix 4A

To explore the relationships between wetland adaptation traits and LES/PES (leaf/plant economics spectrum) traits in our manuscript, we collated published and unpublished data from 5 sources, including three sources having an experimental setup, and two describing a field study. Here, we provide (1) more detailed descriptions of the data in these studies, and (2) show how different experimental/field settings have effects on the both wetland adaptation traits and LES/PES traits, using box plots and scatter plots.

We assume that individual wetland plants develop their adaptation strategies to the environment, independent of whether the exposure happened in the field or at experimental conditions. For our proof-of-principle exploration on the relationships between adaptation traits and LES/PES traits, we focused on three pairs of relationships for which we had concomitant and sufficient data (root porosity vs. leaf nitrogen (N), radial oxygen loss (ROL) vs. leaf N, iron tolerance vs. specific leaf area, SLA). Root porosity and ROL are two very important ecophysiological adaptation traits at flooded conditions (Visser *et al.*, 2000b; Colmer, 2003b; Voesenek & Bailey-Serres, 2015), and reduced iron and other reduced toxins is considered as the cause of the absence of non-wetland plants in wetland conditions (Snowden & Wheeler, 1993). Leaf N and SLA are leading traits driving the LES/PES axis (Wright *et al.*, 2004; Diaz *et al.*, 2016).

To evaluate the effects of experimental/field setting on traits, we can directly relate categorical fertility/water regimes/toxicity/light availability treatments with the wetland adaptation traits (root porosity and ROL) and leaf N for the experimental studies.

For the field studies, we do not have direct measurements of environmental conditions. Instead, we evaluate each species' Ellenberg indicator value for nitrogen (Ellenberg N) as well as leaf N (at both at individual and species level) against the habitat types to indirectly show the fertility differences between habitats and how different habitats drive the leaf N.

The full range of the Ellenberg N is from 1 (least) to 9 (excessive supply); the most intensive survey of the global vascular plants' leaf N database reported the range between 2.48 to 68.98 mg/g (Diaz *et al.*, 2016). Our data showed an almost full range of Ellenberg N and leaf N (Fig. 4S3A to Fig. 4S3C below) suggesting our data is representative of the wide range of habitat fertility. Similarly, the variability of Ellenberg N and leaf N within each habitat type also reflects a fertility gradient within habitat types in our research data.

Experimental studies:

1. Dataset from van Bodegom *et al.* 2008

In this study, six species from wet dune slacks were selected to represent different habitat fertility and wetness. All individuals were trimmed and then moved to greenhouse for one week's acclimatization.

Then four treatments with six replicates each were established in a 2*2 factorial design. The two waterlogging treatment were saturated (with standing water layer of 0-1 cm) and inundated (with standing water layer of 10 cm). The two soil oxygen demand (SOD) factors are increased SOD or no added SOD. After nine weeks, the plants were harvested and root porosity and leaf N were measured. Fig. 4S1A to Fig. 4S1C describe how the water regime (flooded and waterlogged) affects root porosity and leaf N and the relationships between the two traits.

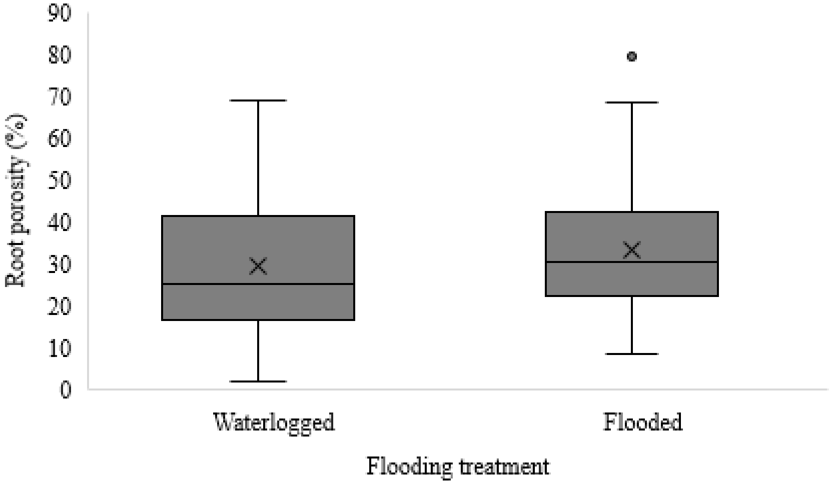

Figure 4S1A Boxplot of individual measurements of root porosity grouped by water regime (n=120).

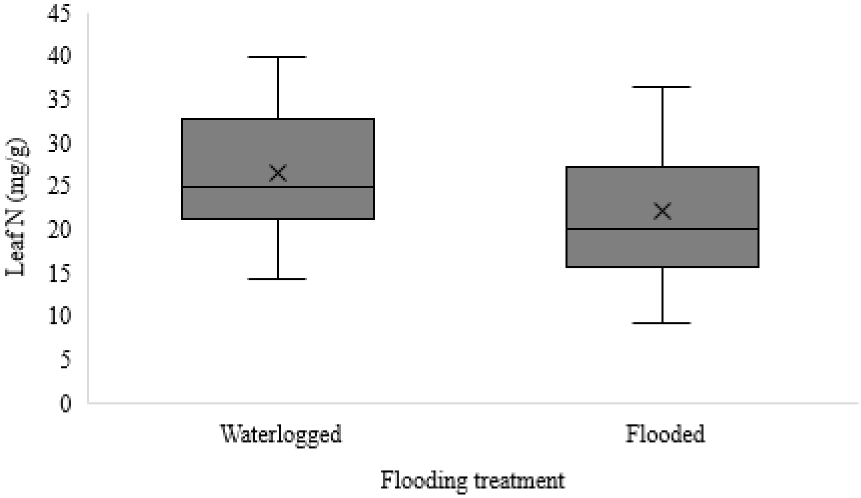

Figure 4S1B Boxplot of individual measurements of leaf N grouped by water regime (n=120).

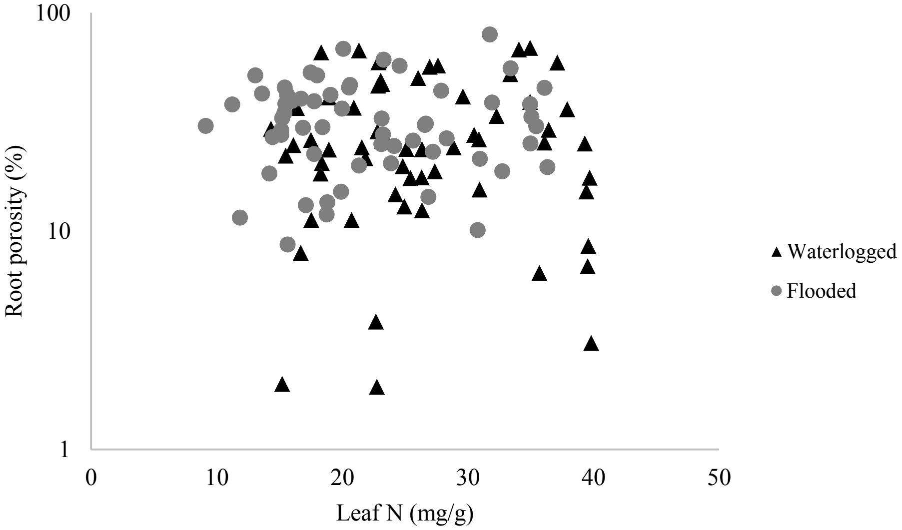

Figure 4S1C The relationships between root porosity and leaf N grouped by water regime (n=120).

2. Dataset from van Bodegom *et al.* 2005

In this study, five species representing pioneer or late-successional dune species living at dry to wet habitat were collected and cultivated in a greenhouse. Under all possible (16) combinations of treatments including light (shaded/full light), fertility (high/low), reduced metal concentrations (high/low) and water regime (waterlogged/field moisture) in six replicates for ten weeks, radial oxygen loss (ROL) and leaf N were determined. The ROL data were heavily zero-inflated (92 out of 209 measurements showed no ROL), so we grouped the ROL data into four classes. The first class contained all 92 measurements of no ROL, and remaining 117 points were evenly divided into the other three classes in the ascending order (39 measurements for each bin).

Class 0: ROL=0 µmol O_2 h^{-1} per g root dry weight, n=92; class 1: ROL=2.5 - 21.5 µmol O_2 h^{-1} per g root dry weight, n=39; class 2: ROL=21.6 - 85 µmol O_2 h^{-1} per g root dry weight, n=39; class 3: ROL=90 - 1212 µmol O_2 h^{-1} per g root dry weight, n=39.

Fig. 4S2A to Fig. 4S2D describe the how fertility (with/without nutrient addition), light availability (light/shade), water regime (flooded and waterlogged), toxicity affect leaf N and the relationships between the two traits. The relationships between leaf N and root porosity are shown in Fig. 4S2E. Fig. 4S2F shows the distribution of ROL classes under the above experimental treatments.

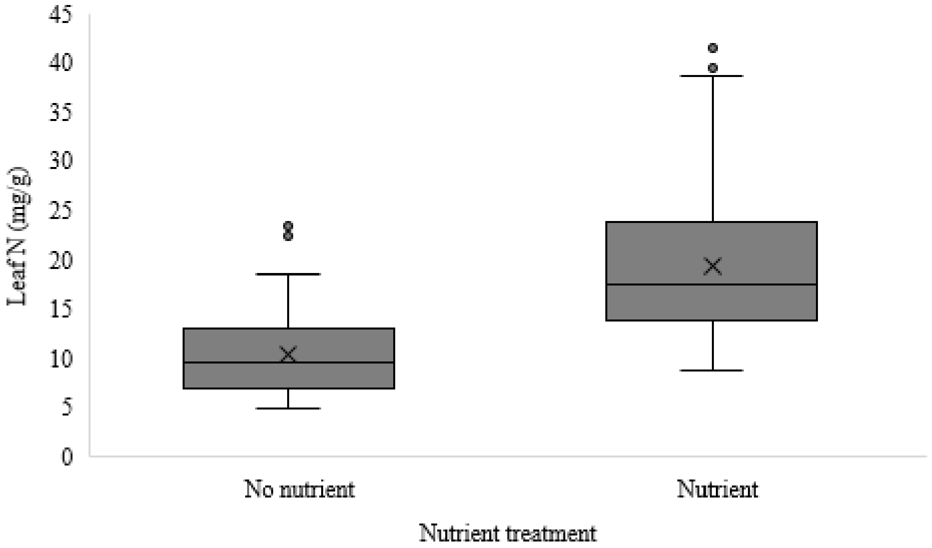

Figure 4S2A Boxplot of individual measurements of leaf N grouped by nutrient addition (n=209).

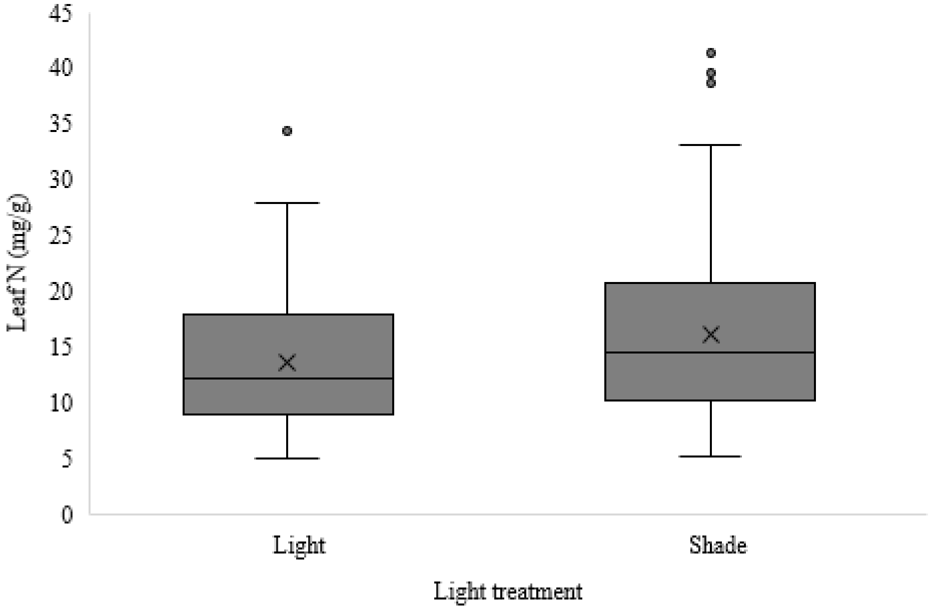

Figure 4S2B Boxplot of individual measurements of leaf N grouped by light availability (n=209).

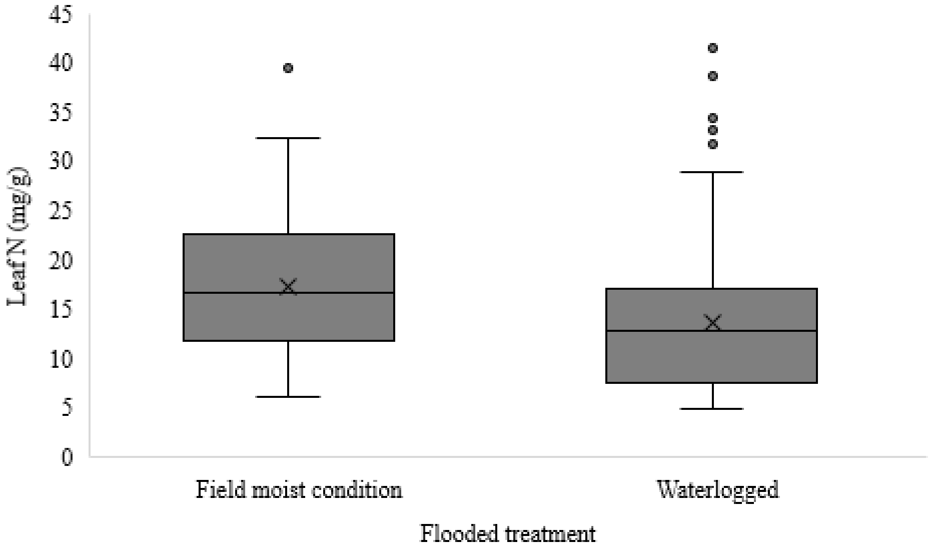

Figure 4S2C Boxplot of individual measurements of leaf N grouped by water regimes (n=209).

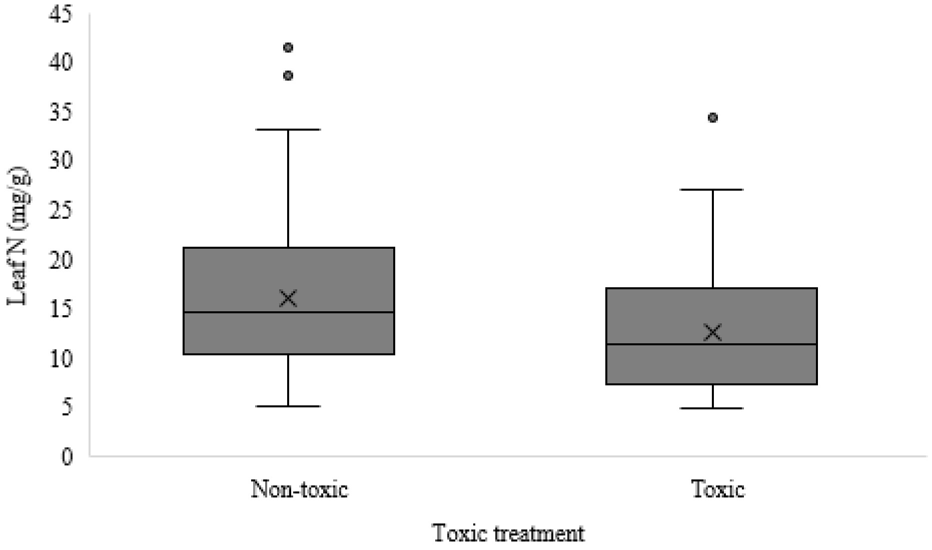

Figure 4S2D Boxplot of individual measurements of leaf N grouped by toxicity treatment (n=209).

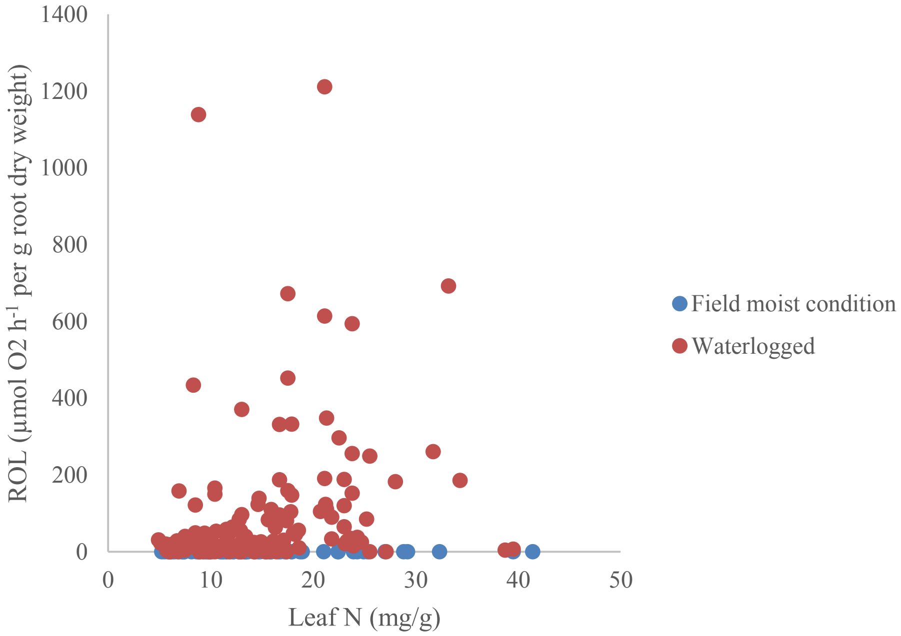

Figure 4S2E The relationships between ROL and leaf N grouped by water regime (n=209).

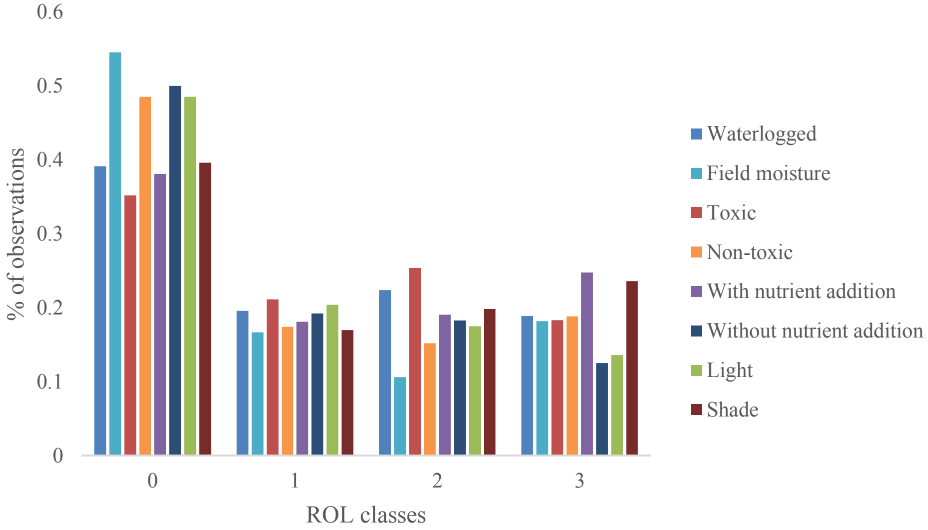

Figure 4S2F The distribution of ROL classes under each treatment.

3. Dataset from Snowden & Wheeler 1993

In this study, seedlings of 43 wetland species native to Britain were cultivated under experimental conditions for two weeks, in 10% Rorison solution containing iron (as ferrous sulphate) at concentration of 3.8 (control), 10, 25, 50,75 and 100 mg Fe/L. The relative growth rate (RGR) was calculated by:

*RGR (per day) = (final dry weight-mean initial dry weight)/ (mean initial dry weight*14)*

The standardized RGR (RGRc) was expressed as a percentage of the RGR to the control treatment. The iron tolerance index was then calculated as the sum of all treatments (except for the control). This index comprises the data available from this study (and can potentially range from 0 to 500%).

Field studies:

4. Dataset from Ukraine unpublished data (Van Bodegom, unpublished a).

In this study, root porosity and leaf N of 53 species from forested/shrub wetlands or marsh habitat were measured in the field. We used each species' Ellenberg indicator for nitrogen (Ellenberg N) and leaf N (both individual and species level) against the habitat types to indirectly show the fertility differences between habitats and how different habitats drive the leaf N (Fig. 4S3A to Fig. 4S3C).

5. Dataset from the Netherlands unpublished data (Van Bodegom, unpublished b).

In this study, 22 species from fen habitat were measured in the field for root porosity and leaf nitrogen. Data is displayed as together with those from field study 4 (Fig. 4S3A to Fig. 4S3C)

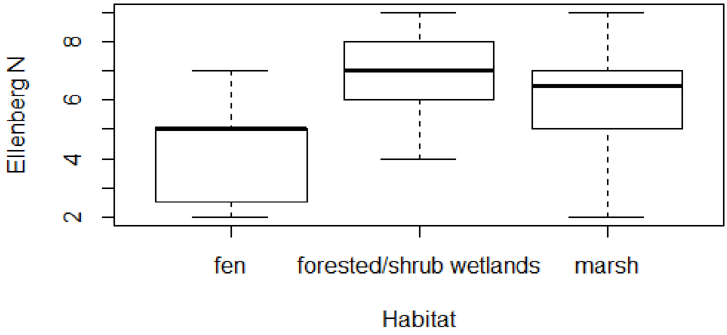

Figure 4S3A Boxplot of Ellenberg N value for species grouped by habitat type (n=40).

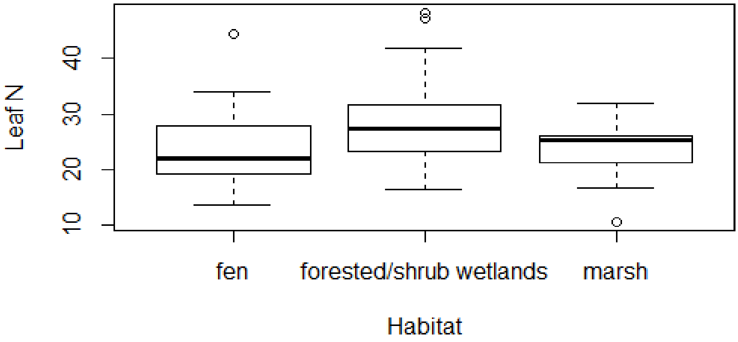

Figure 4S3B Boxplot of mean species' leaf N grouped by habitat type (n=83).

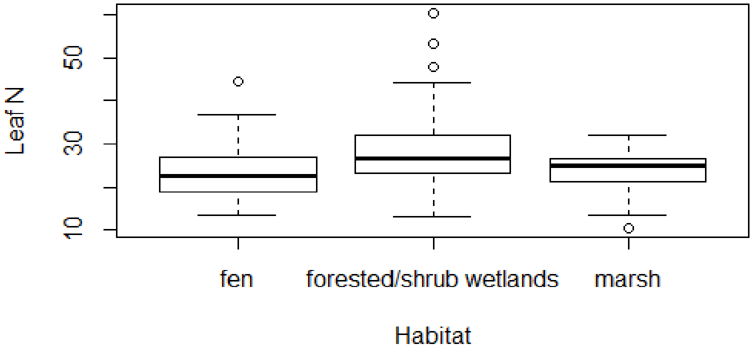

Figure 4S3C Boxplot of individual measurements of leaf N grouped by habitat type (n=147).