Received: 5 May 2021 | Accepted: 13 December 2021
DOI: 10.1111/1365-2435.14001

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Disentangling the roles of plant functional diversity and plaint traits in regulating plant nitrogen accumulation and denitrification in freshwaters

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Funding information
Boliden Minerals AB, Grant/Award Number: 2013-03325 and 2014-01134; VINNOVA; LKAB

Handling Editor: Verónica Ferreira

Abstract

1. There is a growing recognition that functional measures of diversity, based on quantification of functionally important species traits, are useful for explaining variation in ecosystem processes. However, the mechanisms linking functional diversity to different processes remain poorly understood, hindering development of a predictive framework for ecosystem functioning based on species traits.
2. The current understanding of how the functional traits of aquatic plants (macrophytes) affect nitrogen (N) cycling by regulating microbial communities and their activity in freshwater habitats is particularly limited. Denitrifying bacteria are typically associated with the roots of both aquatic and terrestrial plants and denitrification is the main cause of loss of N from ecosystems. Disentangling the interplay between plants and microbial denitrifiers is key to understanding variation in rates of denitrification from local to landscape scales.
3. In a mesocosm experiment, we varied the species richness (monocultures or two-species mixtures) and composition of macrophytes. We quantified effects of both macrophyte functional diversity, quantified as functional trait dissimilarity, and functional trait composition, quantified as community weighted mean trait values, on N removal in wetlands. We used structural equation modelling to disentangle the direct and indirect influences of traits on N accumulation in plant biomass, denitrification activity and abundance of key bacterial denitrification genes (*nirS* and *nirK*).
4. Both functional diversity and functional trait composition regulated N removal, explaining 70%–94% variation in the underlying ecosystem processes. Increased macrophyte functional diversity increased plant N accumulation, and indirectly enhanced denitrification by increasing denitrification gene abundance. Among traits, greater plant relative growth rates, specific leaf area and above-ground

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biomass increased plant N accumulation. Denitrification activity increased with increasing below-ground biomass but decreased with increasing root diameter.

5. These findings improve our understanding of N removal in freshwater wetlands dominated by macrophytes, and have broad ecological implications for wetland management targeting enhanced ecosystem services. Our results highlight the potential for optimizing denitrification and plant N accumulation in wetlands and thereby improving water purification by increasing macrophyte functional diversity and ensuring the presence of key traits in macrophyte assemblages.

KEYWORDS
biodiversity, denitrification, ecosystem functioning, ecosystem service, functional diversity, functional traits, macrophytes, plant uptake

1 | INTRODUCTION

Ecosystem services that support human societies are regulated by the activities of multiple species that often link across habitat and ecosystem boundaries (Kareiva et al., 2007; Kremen, 2005). For example, denitrification, a key ecosystem service, involves the reduction of nitrate to gaseous nitrogen compounds by micro-organisms typically associated with the roots of both aquatic and terrestrial plants that results in removal of N from soils, sediments and aquatic habitats to the atmosphere (Gagnon et al., 2007; Salvato et al., 2012; Wu et al., 2017). The land–wetland–river continuum retains 70% (101 Tg N/year) of the anthropogenic reactive N added to the continental biosphere (Billen et al., 2013) and wetlands can retain 64% of the total nitrogen (TN) load (Saunders & Kalff, 2001), thereby acting as a sink of global N (Mitsch et al., 2013). The global denitrification potential in wetlands is estimated as 18 Tg N/year (Jordan et al., 2011). Significant nitrogen is also removed from wetland waters through plant nitrogen accumulation (Verhoeven et al., 2006; Vymazal, 2007; Zedler, 2003).

Although denitrifying bacteria generally thrive in the vicinity of plant roots (Moreau et al., 2015), plants can also inhibit the activity of N transforming micro-organisms through root exudation and modification of N uptake rates (Hu et al., 2018; Moreau et al., 2019). It is well recognized that the diversity and activity of soil microbial communities in general can be influenced by individual plant species (Harrison & Bardgett, 2010; Wardle et al., 2003). However, the role of plant diversity in regulating denitrification and other N-cycling processes (Cantarel et al., 2015; de Vries & Bardgett, 2016; Moreau et al., 2015) remains poorly understood, especially in aquatic habitats, hindering prediction of the potential consequences of plant biodiversity for improved N removal and water purification. Disentangling the interplay between plants and microbial denitrifiers is key to understanding variation in rates of denitrification from local to landscape scales.

The influence of individual plant species on the ecosystem processes underpinning N cycling, including above-ground primary production, plant nutrient uptake and leaf litter decomposition are linked to their particular ‘functional traits’ (Cadotte, 2017; Craine et al., 2002; de Bello et al., 2010; Frainer & McKie, 2015; Lavorel et al., 2011; Mokany et al., 2008), comprising key attributes of species' phenotypes that regulate their influences on ecosystem functioning (Díaz et al., 2007). For example, traits of plant roots, such as root diameter and root N concentration, and of whole plants, including relative growth rate and nitrogen use efficiency, control not only nutrient uptake rates by the plants themselves, but also the abundances of denitrifying bacteria and potential nitrification and denitrification activities in soils and sediments (Cantarel et al., 2015; Legay et al., 2014; Moreau et al., 2015). Previous studies have addressed the importance of incorporating plant functional traits into our understanding of ecosystem functioning (Cadotte, 2017; Díaz & Cabido, 2001; Lavorel et al., 2011), but experimental demonstrations of how both the composition and diversity of traits influence key functions, such as those underpinning N cycling in aquatic systems, are still rare.

Increasing diversity of plant species traits might improve ecosystem functioning rates by enhancing the potential for the so-called ‘trait complementarity’, whereby the combined outcome of multiple traits interacting together leads to an increase in ecosystem process rates (Loreau & Hector, 2001). For example, overall N uptake rates might be higher in a community characterized by a greater diversity of root structures penetrating to different depths in a sediment profile, compared with a species monoculture. Increasing plant functional diversity might also facilitate the activity of micro-organisms involved in N-cycling (De Deyn et al., 2009; Fornara & Tilman, 2008) by regulating the diversity of habitats available for microbes, and the quantity, quality and diversity of resources (De Deyn et al., 2004, 2011; Eisenhauer et al., 2010). Habitat diversity was recently shown to be a driver of denitrifier diversity and abundance in sediments (Wittorf et al., 2020). In wetlands, denitrification activity is largely controlled by aquatic plants (macrophytes) (Ruiz-Rueda et al., 2009), although the mechanistic understanding of how macrophyte community composition and diversity, and especially functional diversity, regulates macrophyte–denitrifier interactions and their outcomes for the N removal service, remains limited.

In a mesocosm experiment, we investigated how the functional diversity of macrophyte traits regulates plant N accumulation, abundances of denitrification genes and associated denitrification rates, and ultimately N removal from water in wetlands. The abundances of denitrification genes were quantified as a proxy for the size of the bacterial denitrifying community representing the genetic potential for denitrification. We varied macrophyte community composition and diversity among mesocosms by selecting species from a pool of twelve, to achieve a gradient in plant functional diversity. We scored our species for a total of 10 plant functional traits, and quantified plant functional diversity based on the functional dissimilarity (FDis) index. We further characterized functional trait composition based on calculation of community-weighted means (CWMs) for a subset of specific traits, to quantify the abundance-weighted concentration of those traits in each assemblage. We then used structural equation modelling (SEM) to disentangle direct effects of functional diversity and specific plant traits on ecosystem processes from indirect effects arising from changes in abundance of denitrifying organisms. We hypothesized that (a) increasing functional diversity will increase N removal from the water, both by enhancing plant N accumulation and the abundance and activity of denitrifying micro-organisms, and (b) that differences in the effect of specific plant functional traits on plant N accumulation and denitrification will lead to differences in the dominant N removal pathways among our experimental assemblages (Choudhury et al., 2018; Hallin et al., 2015).

2 | MATERIALS AND METHODS

2.1 | Experimental design and setup

We conducted a 100-day greenhouse mesocosm experiment, using 12 macrophyte species collected from wetlands near Uppsala, Sweden in the hemiboreal vegetation zone (Ahti et al., 1968). The selected species represented three of the major growth forms of macrophytes—emergent, submerged and bryophytes (see Table S1) previously shown to differ in their nutrient acquisition strategy and associated N removal rates from water (Barko et al., 1991; Choudhury et al., 2018; Madsen & Cedergreen, 2002; Sculthorpe, 1967). These species occur naturally in temperate, boreal and sub-arctic regions (Hallingbäck & Holmåsen, 1985; Hedenäs & Hallingbäck, 2014; Mossberg & Stenberg, 2003).

Macrophytes were grown in monocultures and two species mixtures (Table S1) in a nested design. In the two species mixtures, growth forms were paired in four possible combinations, that is, emergent–emergent, submerged–submerged, emergent–submerged and emergent–bryophyte (Table S1). Rather than replicate every possible species assemblage, we chose our species assemblage treatments to reflect the natural co-occurrence of the species and growth forms. In total, there were 32 individual species assemblages (Table S1) that were replicated four times resulting in a total of 128 planted mesocosms.

The macrophyte species were planted in 15 L mesocosms containing 9 L nutrient-amended water and 3 L sieved sediment. The initial plant biomass for individual species in each species combination is provided in Table S1. N- and P-free nutrient solution was prepared according to Smart and Barko (1985) and KNO_3 and KH_2PO_4 were added to the water to maintain a concentration of 20 mg NO_3-N/L and 0.01 mg PO_4-P/L, respectively. Nitrate (NO_3–N) was used as a main form of N enrichment in this experiment, in line with our focus on denitrification. In order to ensure availability of copious sediment N, we used sediment contaminated by undetonated ammonium-nitrate-based explosives in mining process water (Herbert et al., 2014). The total N concentration in the sediment was 0.77 ± 0.05 (*SD*) g N/kg-dry weight (*M* ± *SD*). The metal concentrations for Cd, Cr, Cu, Pb and Zn were 0.01 ± 0.01, 42.80 ± 8.10, 32.18 ± 4.77, 3.00 ± 0.51 and 19.36 ± 1.50 mg/kg-dry weight (*M* ± *SD*), respectively. These levels are below the reference value for contaminated soil in Sweden (Naturvårdsverket, 2009, 2016) and the values that can be found in sediment of rivers as well as natural and constructed wetlands (Johnson et al., 2013; Knox et al., 2006; Wang et al., 2014). In total, there were 527 mg N per mesocosm during the whole experiment, considering both nutrient addition and amount in sediment.

2.2 | N concentration in plant biomass

Plants were harvested from each mesocosm at the end of the experiment and sorted into species. The biomass for each species was then divided into above- and below-ground components (except bryophytes which lack below-ground growth), which were then dried separately at 50°C for 7 days and weighed for dry weight determination. Carbon and nitrogen analyses of above- and below-ground biomass were undertaken at the Forest Research Lab, Farnham, UK, according to reference method ISO 10694 & 13878. These data were used to calculate C:N in above- and below-ground biomass at the end of the experiment. Finally, bulk N accumulation ($g\ N\ m^{-2}\ day^{-1}$) in above-ground plant biomass at the end of 100 days of experimental growth in relation to the surface area of the mesocosms was calculated according to Choudhury et al. (2018).

2.3 | Potential denitrification activity

We measured substrate-induced potential denitrification activity (PDA) of macrophyte-associated micro-organisms at the end of the experiment using the acetylene inhibition technique without chloramphenicol (Pell et al., 1996). PDA was measured on roots of emergent and submerged macrophytes and on shoots of bryophytes at the end of the experiment (for details, see Appendix S1, Methods section). Bulk PDA per surface area of mesocosm was calculated by direct measurement of root biomass of all emergent species and only *Hippuris vulgaris* for submerged species at the end of the experiment. For submerged species *Elodea canadensis*, we calculated the root dry weight biomass using the formula, root biomass = 0.000125 + (0.1420187 × shoot biomass) (Tattersdill et al., 2017) and for *Myriophyllum alterniflorum* we used the root:shoot ratio of 0.18 based on Spierenburg et al. (2010). For bryophytes and *Ceratophyllum demersum*, bulk PDA was calculated based on total shoot biomass at the end of experiment since these species do not possess root biomass.

2.4 | DNA extraction and quantitative PCR

To quantify bacterial denitrification gene abundances associated with the roots of the emergent and submerged species and on the shoots of the bryophytes, DNA was extracted from four plants of each species in each mesocosm sampled at the end of the experiment. DNA was extracted from 0.02 to 0.06 g freeze-dried plant material using the MP Biomedical FastDNA® Spin Kit for Soil following the manufacturer's instructions. The extracted DNA was quantified using the Qubit® fluorometer (Life Technologies Corporation). Quantitative real-time PCR (qPCR) based on SYBR green detection was used to quantify the denitrification genes *nirS* and *nirK*, coding for the two different nitrite reductases in denitrification, according to Hellman et al. (2019) (for details see Appendix S1, Methods section).

2.5 | Plant functional traits and strategies related to N-cycling

In total, 10 macrophyte traits were selected to investigate relationships between the composition and diversity of macrophyte traits and the key ecosystem processes underpinning N-cycling *viz*. plant N accumulation, potential denitrification rates, as well as the abundance of bacterial denitrification genes. The selected plant traits were as follows: (a) relative growth rate (RGR), (b) specific leaf area (SLA), (c) specific root surface area (SRSA), (d) C:N in above-ground biomass at the end of experiment, (e) C:N in below-ground biomass at the end of experiment, (f) average root diameter (RD), (g) percentage of fine roots area in relation to total root surface area, (h) allelopathic potential of species, (i) above-ground biomass at the end of experiment and (j) below-ground biomass at the end of experiment. These traits are described in full, with justification for their selection as functional traits as well as trait measurement procedures in Table S2. Besides plant traits, we also investigated Grime's CSR (C, competitive; S, stress tolerant; R, ruderal) strategies (Grime, 1974) and Ellenberg N indicator values (Ellenberg, 1974), to test their relationship with N-cycling (see Table S3 for description and justification for use).

2.6 | Trait-based measures

We employed two indices to quantify the functional characteristics of our macrophyte assemblages, based on the plant traits described above. First, we characterized variation in functional trait composition through quantification of community-weighted mean (CWM) trait values for each assemblage (Garnier et al., 2004). CWM was calculated using the function dbFD in the R package FD (Laliberté et al., 2015), which generates a trait matrix where traits are weighted by the abundance (measured by total end biomass of each species in this study) of all species sharing it. This matrix was then resolved using a principle component analysis (PCA) with the rda function (R package VEGAN; Oksanen et al., 2011) to obtain the axis (component) that explained most of the variation in functional trait composition across the assemblages. PCA analyses were conducted separately on the trait matrix of 10 selected plant traits as well as on the matrix including all traits, Grime's CSR strategies and environmental preference (i.e. Ellenberg N indicator values).

We quantified functional diversity of our macrophyte assemblages using the functional dispersion (FDis) index, available through the function dbFD in the R package FD (Laliberté et al., 2015). FDis measures functional trait distribution in the community accounting for the dissimilarity among traits where a higher value indicates a higher evenness of more dissimilar traits (Laliberté & Legendre, 2010). For each assemblage, we first obtained the centroid in the multivariate space calculated from the trait-based distance matrix where species were weighted by their abundance (measured by total end biomass of each species in this study). Then, the distance between the centroid and each species was calculated and weighted again by their abundance. FDis for each community was then calculated as the sum of these distances (Laliberté & Legendre, 2010).

3 | STATISTICAL ANALYSES

We used SEM to disentangle direct and indirect pathways that we hypothesized would explain variability in N removal from mesocosms. SEM is used to statistically evaluate a series of dependent relationships through the analysis of covariance (Grace & Pugesek, 1997) and allows partitioning causal pathways in complex data (Grace et al., 2010). In our SEMs, we used macrophyte FDis and CWM as surrogates for functional diversity and species trait composition, respectively. We tested for the effect of macrophyte functional diversity and trait composition, fitted as exogenous variables (i.e. variables that are not affected by other variables in the model), on three endogenous variables (i.e. variables whose values are determined by one of the functional relationships in the model): total N removal in mesocosms by (a) plant N accumulation, (b) potential denitrification rates and (c) bacterial denitrification gene abundance (i.e. sum of *nirS* and *nirK* abundances). Data were log-transformed, when necessary, to meet the assumptions of normality and to avoid non-constant error variance. SEM was conducted using the R package LAVAAN (Rosseel, 2012). Model fit was assessed using the root mean square error of approximation (RMSEA) and Chi-square goodness-of-fit index (GFI) (Grace & Pugesek, 1997; Hair et al., 2010).

Following our SEM analyses, we constructed generalized linear models (GLMs) with stepwise regression based on Akaike information criteria (AIC) (Quinn & Keough, 2002) to identify the set of plant traits that best explained plant N accumulation and denitrification rates. For plant N accumulation, initially we selected the CWM for each of the following plant traits: RGR, SLA, SRSA, above-ground biomass at the end of experiment and C:N in above-ground biomass at the end of experiment as predictor variables (see Table S2 for trait justification). For denitrification rates, we selected the CWM for each of the traits: SRSA, RD, below-ground biomass at the end of experiment, C:N in below-ground biomass at the end of experiment and allelopathic potential of species as predictor variables (see Table S2 for trait justification). Data were log-transformed, when necessary, to meet the assumptions of parametric tests. Multicollinearity among predictor variables was assessed for each model based on the variance inflation factor, which was always well below 10 (range: 1.04–2.22), the cut-off for identifying strong autocorrelation (Quinn & Keough, 2002) (see Table S6 for correlation among statistics). GLM analyses were performed using the software IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp).

To investigate the relative importance of potential N removal pathways in macrophyte communities with different growth form assemblages, we studied the relationship between the plant N accumulation and potential denitrification rates for all 32 species assemblages (see also Hallin et al., 2015).

4 | RESULTS

4.1 | Functional trait composition

The first axis (PC1) of the principal component analysis (PCA) of the species trait matrix explained 57.5% of the variation among wetland mesocosms and was positively associated with root diameter, the presence of allelopathy and specific leaf area, and negatively associated with percentage of fine roots area, C:N in below-ground biomass at the end of experiment, below-ground biomass at the end of experiment, specific root surface area, relative growth rate, C:N in above-ground biomass at the end of experiment and above-ground biomass at the end of experiment (Figure 1). The second principal component (PC2) was positively associated with specific leaf area and negatively associated with the presence of allelopathy (Figure 1). PC2 explained 20% of the variation among the wetland mesocosms. PC1, hereafter 'functional trait composition' was used as a predictor variable in subsequent SEM. For associations between plant traits, Grime's CSR strategies and Ellenberg N indicator values, see Figure S1.

4.2 | Structural equation modelling of direct and indirect mechanisms regulating net nitrogen removal

The SEM explained 62% of variation in total N removal from the water, with good model fit ($\chi^2 = 4.03$, $df = 4$, $p = 0.401$; RMSEA = 0.008, $p = 0.554$) (full model output is available in Table S4). Total N removal increased with both increasing plant N accumulation ($r = 0.55$) and denitrification ($r = 0.17$) (Figure 2, see Table S4). Bacterial denitrification gene abundances (sum of *nirK* and *nirS*) indirectly affected net N removal by increasing potential denitrification rates ($r = 0.31$, $p < 0.001$). Variation in functional trait composition affected both plant N accumulation ($r = -0.41$) and denitrification ($r = -0.33$) (Figure 2, see Table S4).

Increasing plant functional diversity had a positive direct effect on plant N accumulation ($r = 0.26$) but its effects on potential denitrification rates were not statistically supported ($r = 0.12$, $p = 0.12$). Finally, a positive effect of functional diversity on denitrification gene abundances was apparent ($r = 0.20$). Functional diversity, trait composition and denitrification gene abundances together explained 70% of the variation in denitrification rates, while functional diversity and functional trait composition explained 72% and 91% of the variation in plant N accumulation and denitrification gene abundances, respectively (Figure 2, see Table S4).

4.3 | Effect of individual traits on N-cycling

The final model for plant N accumulation included the traits relative growth rate, specific leaf area and above-ground biomass at the end of experiment (adjusted $R^2 = 0.62$). We found a positive association between plant N accumulation and relative growth rate, above-ground biomass and specific leaf area (Table 1). Potential denitrification rates were best explained by the root diameter and below-ground biomass at the end of experiment (adjusted $R^2 = 0.19$). Denitrification rates were negatively associated with root diameter and positively associated with below-ground biomass at the end of experiment (Table 1).

4.4 | Potential N removal pathways

The importance of denitrification and plant N accumulation for N removal differed markedly between the species assemblages (Figure 3; see Table S5). In general, monocultures of submerged macrophytes and mixed cultures of submerged–submerged macrophytes had plant uptake as the main N removal pathway (Figure 3). By contrast, in monocultures of emergent species and bryophytes, both N accumulation and denitrification were important removal pathways, though their relative importance varied among species (Figure 3; Table S5). For example, in monocultures of *Equisetum fluviatile*, denitrification was the main removal pathway, whereas N accumulation was the main removal pathway for monocultures of *Phragmites australis*. Among bryophytes, denitrification was the main pathway for *Fontinalis antipyretica* and *Leptodictyum riparium*, whereas N accumulation was the main pathway for monocultures of *Sphagnum fallax* (see Table S5). In mixed cultures, denitrification tended to be the main N removal pathway for emergent–bryophyte assemblages, while both pathways were important for emergent–emergent assemblages (Figure 3; Table S5).

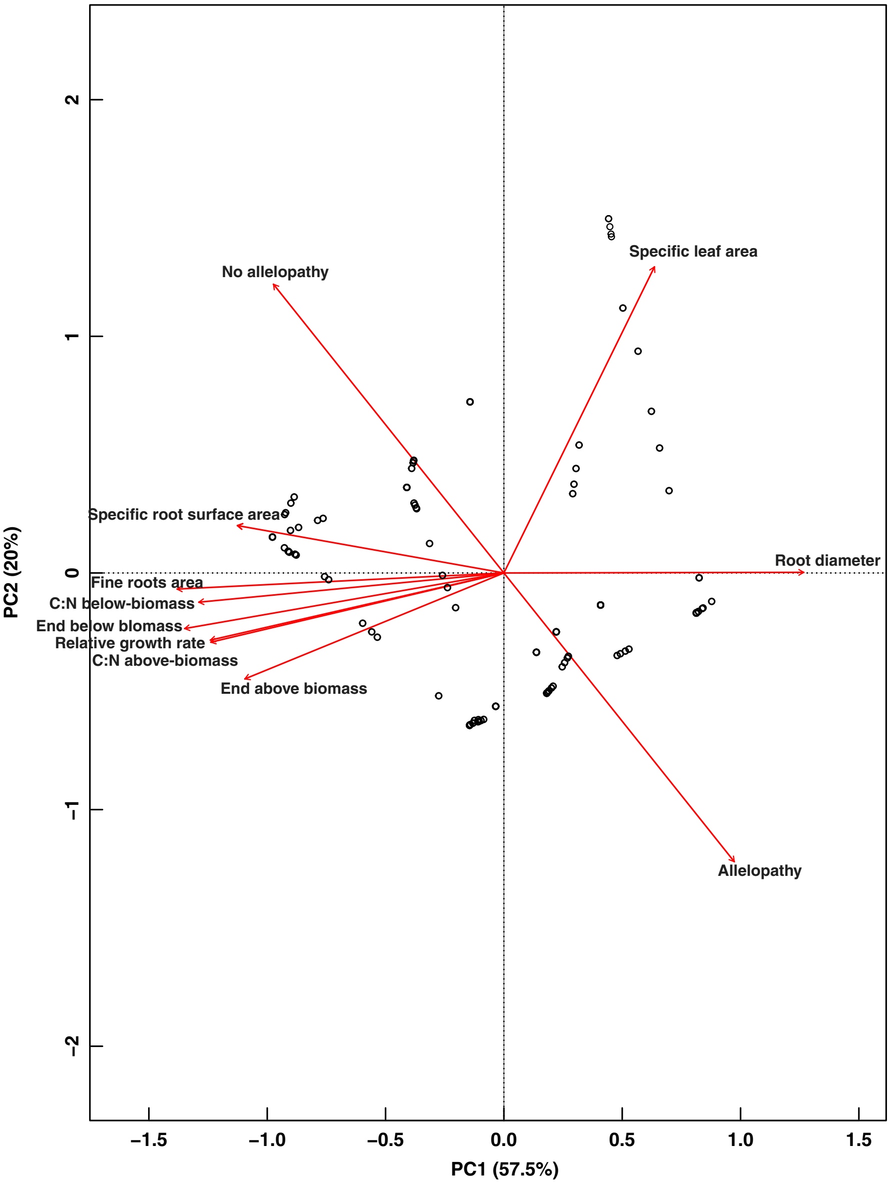

FIGURE 1 Principal component analysis (PCA) of the functional trait composition of the macrophyte assemblages, weighted according to their relative abundance, calculated as the community-weighted mean (CWM) trait values. Dots represent individual mesocosms and arrows represent variables measured in individual mesocosms. End above and below biomass refers to above-ground and below-ground plant biomass at the end of the experiment, respectively. C:N above-biomass and C:N below-biomass refer to C:N ratios in above- and below-ground plant biomass at the end of experiment, respectively

5 | DISCUSSION

Our study disentangles the roles of key plant traits in macrophyte communities versus overall functional diversity for denitrification and plant N accumulation. These processes are central for water purification provided by wetlands, which together contribute to the removal of bioavailable N from wetland surface water. Influences of macrophyte traits and functional diversity on these N removal processes occurred by both direct and indirect causal pathways. For example, functional diversity had a direct positive effect on plant N accumulation, while the direct effects of increasing root diameter and allelopathy were negative. By contrast, a positive effect of functional diversity on denitrification was mediated through a positive effect on bacterial denitrification gene abundances. These findings emphasize the importance of both protecting and rehabilitating the diversity of macrophytes in wetlands, which are among the world's most threatened ecosystems (Brinson & Malvarez, 2002; Gibbs, 2000), and demonstrate the value of using information on the composition and diversity of species traits for understanding variation in key ecosystem processes underpinning ecosystem service delivery.

In agreement with our first hypothesis, functional diversity influenced plant N accumulation and denitrification rates. We quantified functional diversity using the functional dissimilarity index, which is higher for communities characterized by a more even distribution of dissimilar functional traits (Laliberte & Legendre, 2010). This is expected to increase the potential for complementary interactions within the community to influence ecosystem functioning (Gessner et al., 2010; McKie et al., 2008), with both complementary resource use and/or facilitation, known to favour higher ecosystem process rates, including plant N accumulation (Frainer et al., 2014; Hillebrand & Matthiessen, 2009). Complementarity in plant nutrient uptake and in total N removal from wetland surface water has been observed previously in wetland studies, but primarily in the assemblages combining emergent macrophytes and bryophytes that differ in

FIGURE 2 Structural equation modelling (SEM) of total nitrogen (N) removal from wetland mesocosms. Solid lines indicate significant ($p < 0.05$) positive and negative relationships, respectively, while dashed lines indicate non-significant ($p > 0.05$) relationships. Standardized correlation coefficients are shown in boxes. The variation explained by response variable is denoted as r^2 in parentheses (Org-N = Organic nitrogen). The community-weighted mean values of traits are based on Principal component 1 (PC1) of the studied functional traits (Figure 1)

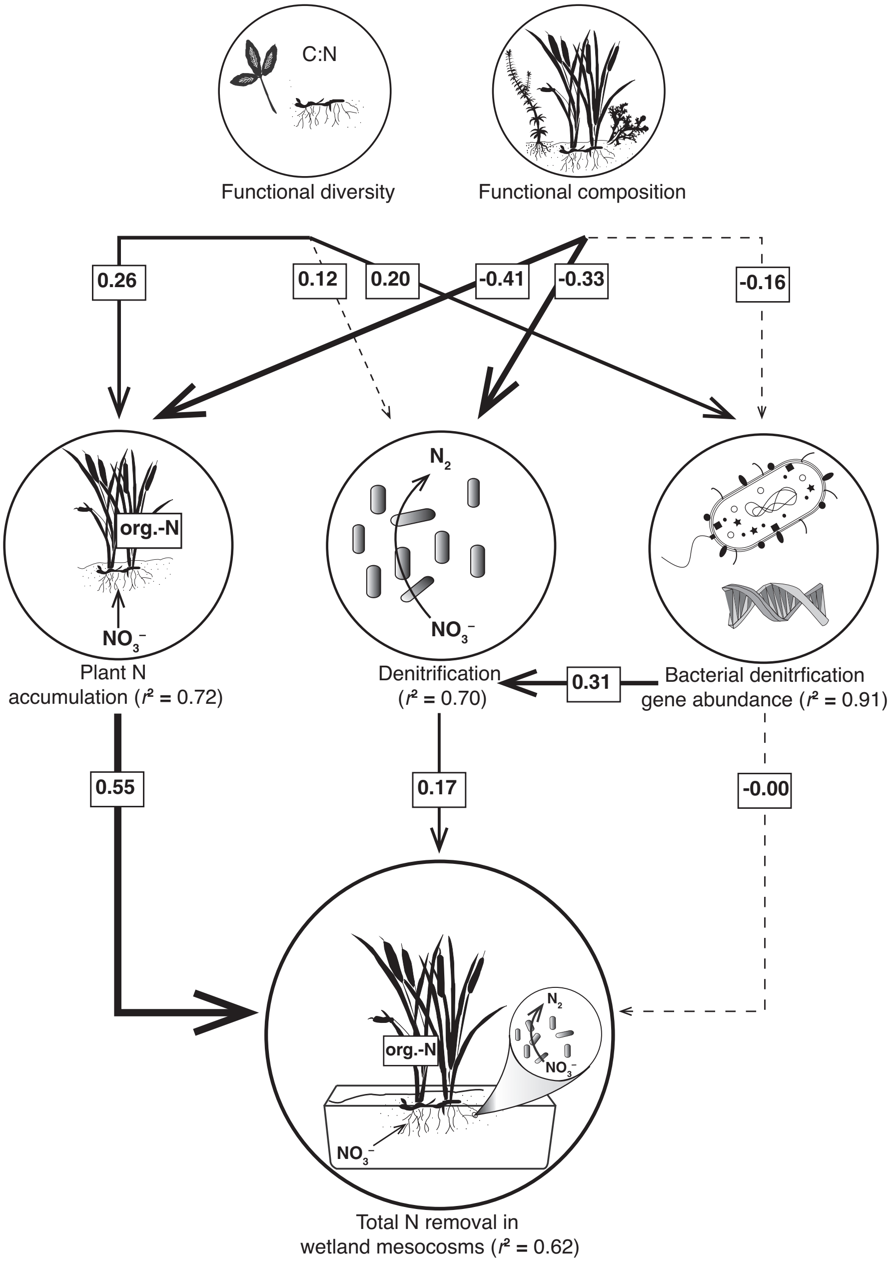

TABLE 1 The best fit generalized linear model (GLM) explaining variation in the plant N accumulation and potential denitrification rates (PDA) as selected by stepwise regression (RD, root diameter; RGR, relative growth rate; SLA, specific leaf area)

Response	Predictor	Standardized coefficients (β)	t	p-value	Partial correlation
Plant N accumulation	RGR	0.57	6.92	0.000	0.53
	SLA	0.34	5.72	0.000	0.46
	Above-ground biomass	0.36	4.54	0.000	0.38
Potential denitrification rates	RD	−0.19	−2.36	0.020	−0.20
	Below-ground biomass	0.36	4.41	0.000	0.37

nutrient acquisition strategy and niche requirements for nutrients (Choudhury et al., 2018; Hallin et al., 2015). In the present study, we further observed that functional diversity had a direct positive influence on bacterial denitrification gene abundances. This increase in denitrifier abundances in turn drove an increase in denitrification rates. Previous studies have focused on the direct relationships between ecosystem processes and functional diversity within an organism guild (Frainer & McKie, 2015). Our

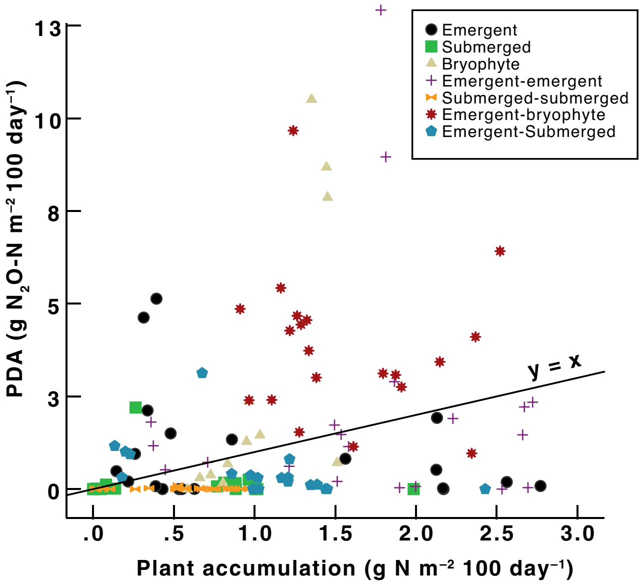

FIGURE 3 Relationship between the two studied potential nitrogen (N) removal pathways, viz. plant N accumulation and potential denitrification rates (PDA) in the wetlands planted with different growth form combinations of macrophytes. Values above the 1:1 line indicate domination of denitrification, whereas values below the line indicate plant N accumulation as potential N removal pathway

findings thus demonstrate that increasing functional diversity in one organism group can influence abundance of another organism group (sensu Eviner & Chapin, 2003), in our case denitrification gene abundances, which had a knock on effect on ecosystem processes including potential denitrification rates.

In addition to functional diversity per se, ecosystem process rates can be strongly regulated by variation in the dominance of particular traits, as characterized by our abundance-weighted measure of functional trait composition (Mokany et al., 2008). In our experiment, plant N accumulation and denitrification rates were regulated by similar suites of plant traits (Figure 1, PC1), although the specific traits which best predicted each ecosystem processes differed (Table 1). Macrophyte communities dominated by a high specific root surface area, fine roots and high relative growth rates also had higher plant N accumulation. Plants with high specific root surface area are typically associated with high nutrient uptake rates, high relative growth rate and high proportion of fine root biomass in terrestrial ecosystems (Cornelissen et al., 2003), and our results similarly indicate that these traits might enhance N accumulation by macrophytes in wetlands. Also in agreement with our results, de Vries and Bardgett (2016) found that higher abundance of grassland species with specific leaf surface area (i.e. high community-weighted mean of specific leaf area) significantly explained N uptake by grasses and herbs. Both terrestrial and aquatic plants have been shown to increase N uptake and/or tissue N concentration and net dry mass production with increasing relative growth rate and specific leaf area, which in turn might also influence above-ground biomass production (Güsewell, 2004; Jampeetong et al., 2012; Osone et al., 2008). The lower N accumulation observed in macrophyte communities characterized by allelopathy and high affinity for N, that is, high Ellenberg N indicator values (Figures 1 and 2; Figure S1), might be due to strong interspecific competition for N by species with these traits, resulting in reduced plant N accumulation and suppressing growth of macrophytes (Gopal & Goel, 1993; Güsewell, 2004).

Below-ground plant traits, that is, root traits and submerged biomass (in case of bryophytes) were the best predictors of denitrification (Table 1). Macrophyte assemblages dominated by species with high specific root surface area (e.g. emergent macrophyte species of *Carex rostrata*, *E. fluviatile*) and high root/submerged biomass at the end of the experiment (e.g. the vascular plant *C. rostrata* and bryophyte species of *F. antipyretica*) resulted in increased denitrification rates. Higher below-ground biomass indicates high root biomass and root surface area, which should generally enhance both surface area and labile dissolved organic carbon (DOC) supply for microbial growth and activity (Hunter et al., 2014; Yang & Crowley, 2000). In a previous study, it was found that bryophyte *Drepanocladus fluitans* can support higher denitrification gene abundances and 2–3 times higher shoot- or thallus-associated potential denitrification rates compared to the roots of emergent macrophytes. This can be attributed to the high specific surface area and labile DOC provided by bryophyte shoots or thallus biomass for bacterial growth and activity, compared with the thicker roots and lower surface area to volume ratio of many emergent macrophytes (Hallin et al., 2015; Turetsky, 2003). Future research should focus on whether other mixtures of bryophyte species as well as submerged leaves of other non-bryophyte macrophytes, particularly those with finer leaf structures, are also associated with significant denitrification. Most intriguingly, macrophyte communities with a higher abundance of plants with allelopathy and high affinity for N showed reduced denitrification rates (Figures 1 and 2; Figure S1). This might reflect competition between plants and denitrifying microbes for N within these macrophyte communities, as previously observed among terrestrial plants (Moreau et al., 2015). Such competition, possibly together with the potential effects of plant secondary metabolites or root exudates associated with allelopathic effects, appears to have negative consequences for denitrifying bacterial activity. In contrast to denitrification rates, the dominant traits in our macrophyte communities did not influence denitrification gene abundances, which were regulated by functional diversity per se. Therefore, it is unlikely that both functional trait diversity and functional trait composition are equal in their influence on the ecosystem processes of plant N accumulation and denitrification (Mokany et al., 2008).

6 | CONCLUSIONS

Our results demonstrate that not only the presence of particular plant traits but also the overall functional diversity of those traits are important for removal of N from surface water in wetlands. This reflects both direct and indirect influence on plant N accumulation, and on the abundance and activity of denitrifying bacteria associated with roots of emergent and submerged macrophytes and on shoots or thallus of bryophytes. In our study, the identity of dominant traits in macrophyte communities had both positive and negative outcomes for ecosystem processes depending on the role of specific traits on measured ecosystem processes, *viz*. plant N accumulation and denitrification. Previously, we demonstrated that macrophyte growth form combinations can guide selection of optimal macrophyte assemblages for N removal from wetlands (Choudhury et al., 2018; Hallin et al., 2015). The current study extends these findings by identifying not only specific plant traits that either enhance or suppress specific N removal pathways, but also quantifying the role of functional diversity per se. Individual plant traits, functional composition (and the dominance of certain plant traits in particular) and their interactions (e.g. direct and indirect competition) were shown to have an important role for N removal in wetlands dominated by macrophytes. These findings have broad ecological implications for management of freshwater habitats targeting enhanced ecosystem services by increasing functional diversity of relevant traits in macrophyte assemblages. In particular, our results demonstrate the potential for identifying macrophyte species and species combinations based on their traits for use in constructed wetlands that support not only plant N accumulation but also denitrification. Our study emphasizes the need for the preservation of a high level of functional diversity in macrophyte communities in wetlands to support efficient N removal.

ACKNOWLEDGEMENTS

This study was financed by VINNOVA (The Swedish Innovation Agency), LKAB and Boliden Minerals AB through the project 'miNing' (grant numbers: 2013-03325 and 2014-01134). We thank Joel Segersten and Maria Hellman for field and laboratory work.

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial conflict of interests to disclose.

AUTHORS' CONTRIBUTIONS

M.I.C., F.E., S.H. and B.G.M. conceived the ideas and designed the methodology; M.I.C., V.H. and J.J. performed the experiment and laboratory analyses and collected the data; M.I.C., B.G.M. and A.F. analysed the data; M.I.C., F.E., S.H. and B.G.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.rbnzs7hcm (Choudhury et al., 2021).

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How to cite this article: Choudhury, M. I., Hallin, S., Ecke, F., Hubalek, V., Juhanson, J., Frainer, A., & McKie, B. G. (2022). Disentangling the roles of plant functional diversity and plaint traits in regulating plant nitrogen accumulation and denitrification in freshwaters. *Functional Ecology*, 36, 921–932. https://doi.org/10.1111/1365-2435.14001