



Functional biogeography: Stoichiometry and thresholds for interpreting nutrient limitation in aquatic plants

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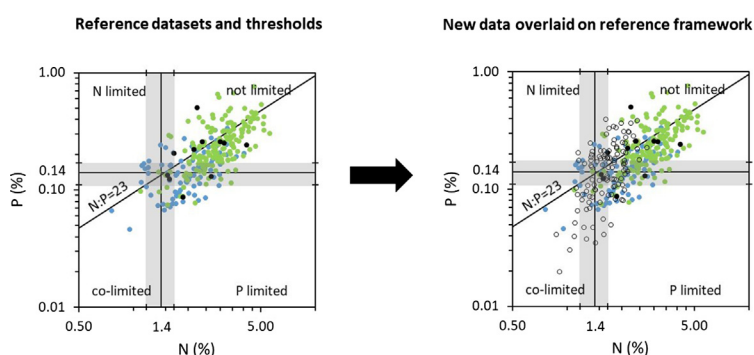
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HIGHLIGHTS

- Nutrient plant tissue stoichiometry is difficult to interpret in the wild.
- Nutrient correlations (sediment, water and plants) along nutrient gradients help.
- Synthesis of nutrient limitation thresholds in aquatic plants helps.
- Comparative analysis in novel framework including reference data helps.
- Novel interpretations of the mass development of an aquatic plant were unravelled.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric N pollution may shift nutrient limitations in aquatic autotrophs from N to P or cause an intensification of P limitation in formerly pristine areas. Small changes in nutrient supply in oligotrophic lakes and rivers could lead to large changes in relative plant growth and yield with possible knock on effects on ecosystem carbon cycling through changes in the decomposition rate of their tissue. Previous biogeographical studies have shown inconsistent responses of plant nutrient tissue content and stoichiometry (functional traits) to external nutrient availability. Here we used a single species, *Juncus bulbosus*, to test the interplay between plant tissue nutrient (content and stoichiometry) and external environmental factors (local and catchment scale). We developed a comparative approach applicable globally to assess the thresholds for nutrient limitation in aquatic plants in the wild. Phosphorus in *Juncus bulbosus* tissue was negatively related to sediment organic matter (Fe root plaque limiting P uptake) and catchment vegetation cover (less P leaching to lakes). Our comparative approach revealed that the lack of increase in N plant tissue along the strong gradient in external N concentration may be explained by P limitation and strict plant tissue N:P ratio. Our comparative approach further showed that the nutrient content and stoichiometry of *Juncus bulbosus* was similar to other submerged aquatic plants growing in nutrient poor aquatic ecosystems. In southern Norway, mass development of *Juncus bulbosus* may be primarily triggered by changes in P availability, rather than CO₂ or inorganic N, as previously thought, although co-limitations are also possible. If so, the mass development of *Juncus bulbosus* in oligotrophic aquatic ecosystems could be an early indicator of increasing P fluxes through these ecosystems which are less limited by N due to high atmospheric N deposition.

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1. Introduction

Post-industrial human activity has magnified the global nitrogen (N) and phosphorus (P) cycles by approximately 100 and 400%, respectively (Falkowski et al., 2000), and N deposition has increased over vast areas in the last decades. This may have shifted nutrient limitations in aquatic autotrophs from N to P or caused an intensified P limitation in formerly pristine areas (Bergström et al., 2005; Elser et al., 2009; Liess et al., 2009; Sardans et al., 2012).

The functional traits nutrient content and stoichiometry of aquatic plants in oligotrophic lakes and rivers may be affected by small changes in nutrient supply and could lead to large changes in relative plant growth (Gerloff and Krombholz, 1966; Chambers et al., 1992), with possible knock on effects on ecosystem carbon cycling through changes in the decomposition rate of their tissue (Enriquez et al., 1993). Oligotrophic lakes in Europe are dominated by isoetids (small rooted plants with basal growth point, high root:shoot ratio) which can be outcompeted by elodeids (submerged rooted plants with apical growth point, low root:shoot ratio) with increasing nutrient supply (Smolders et al., 2002; Sand-Jensen et al., 2018). To which extent aquatic plant tissue content will respond to nutrient supply in the wild and over large geographical areas remains unsettled, and the size effect (regression slope) of that response varied widely between studies (cf. e.g. Kern-Hansen and Dawson, 1978; Robach et al., 1996; Thiébaud, 2005; Demars and Edwards, 2007b). Much of the unexplained variance around the response was explained by taxonomic identity (species effect) in studies with multiple species (Demars and Edwards, 2007b; Frost and Hicks, 2012).

Species nutrient content has been correlated to the species external nutrient optimum (Demars and Trémolières, 2009) and the species turnover along the nutrient gradient has been suggested as a mechanism to changing aquatic plant community stoichiometry (Frost and Hicks, 2012). The change in nutrient tissue content may not be reflected by changes in relative growth rate or maximum yield pass a critical nutrient threshold (Gerloff and Krombholz, 1966; Demars and Edwards, 2007b). The critical nutrient thresholds and critical N:P ratios are likely to be species specific (Gerloff, 1975) and dependent on other environmental conditions, such as pH (Grisé et al., 1986), dissolved CO₂ (Titus and Andorfer, 1996; Madsen et al., 1998) and temperature (Ventura et al., 2008). It has been shown theoretically that the relative growth rate should be linearly related to N, quadratically to P and unimodal for N:P (Ågren, 2004). Factors affecting the relative growth rate (e.g. temperature, light) and interacting with nutrient supply precluded to explain with simple rules changes in plant stoichiometry (Ågren, 2004; Ventura et al., 2008). Judged from this, it is perhaps not surprising that nutrient and temperature based hypotheses explained little of the observed variability in C, N, P tissue nutrient content and C:N:P ratios in biogeographical studies (e.g. Demars and Edwards, 2007b; Xia et al., 2014; Gong et al., 2018; Wang et al., 2018).

Here we used a single species, *Juncus bulbosus* L., to test the interplay between plant tissue nutrient (content and stoichiometry) and external environmental factors. We used the strong gradient in atmospheric N deposition present in southern Norway (Hessen et al., 2009) to test for a possible intensification of P growth limitations in *Juncus bulbosus*, lower P content where P is limiting, and correspondingly elevated N:P ratios (Elser et al., 2009; Sardans et al., 2012).

More specifically we set out to (i) test whether *Juncus bulbosus* C, N, P and C:N:P in lakes can be related to external environmental conditions (notably nutrient concentrations in water and sediment) and catchment scale descriptors (notably atmospheric N deposition); and (ii) develop a comparative approach applicable globally to assess the likelihood of nutrient limitation in aquatic plants in the wild.

2. Methods

2.1. *Juncus bulbosus* in north-west Europe

Juncus bulbosus is a perennial species known to have low N and P tissue nutrient content (Rørslett, 1988; Roelofs et al., 1994; Samecka-Cymerman and Kempers, 2001). Whether the low plant tissue contents are due to environmental conditions or evolutionary constraints (Juncaceae family) is not known. *Juncus bulbosus* is common in oligotrophic and ultra-oligotrophic European lakes and rivers (Rørslett, 1987; Murphy, 2002). It can grow from terrestrial to totally submerged, and in the most acidic water with pH ≤ 3 (Fyson, 2000) and uncharacteristically in some calcareous turloughs in the Burren (western Ireland, Preston et al., 2002). It has caused problems with mass development along the littoral zones of many lakes and rivers since the mid-1980s (Roelofs, 1983; Aulio, 1987; Svedäng, 1990). Although these problems have receded in some areas (Brandrud, 2002; Lucassen et al., 2016), it remains an issue in southern Norway (Moe et al., 2013; Schneider et al., 2013).

2.2. Collection of *Juncus bulbosus* in lakes of southern Norway

We sampled *Juncus bulbosus* from 112 lakes in southern Norway (Fig. 1), during summer 2007 (24 August–26 September), covering most of the variation in environmental conditions in the area, notably a gradient of atmospheric N deposition (see maps in Hessen et al., 2009). We picked a single random shoot per lake, in the littoral zone, from the dominant type of *J. bulbosus* growth form. The within site variability in C, N, P was calculated from a preliminary study (Kaste et al., 2007) where three to ten shoots per lakes were collected in six lakes at two dates during the growing season (June and September 2006). The within site coefficient of variations (standard deviation/mean) were C = 4%, N = 13%, P = 28%, similar to what was reported by Rørslett (1988) for two rivers and a lake, both studies from southern Norway.

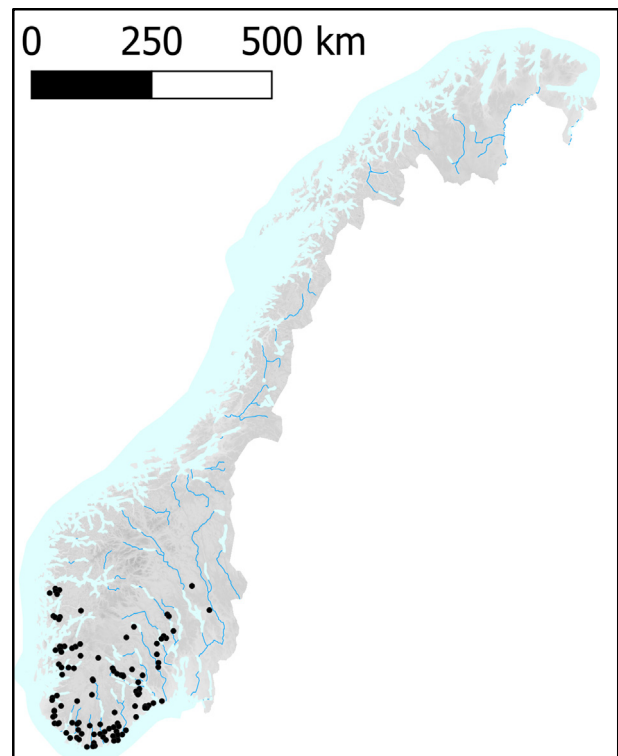


Fig. 1. Site distribution in Norway: 112 lakes.

Table 1

Environmental variables for 106 (water column) and 88 (sediments) of the 112 lakes surveyed during summer 2007, southern Norway.

Environmental variables	Unit	Median	Mean	Range	n	Transformation
Coordinates UTM North and East	Decimal degree	–	–	–	106	None
Electric conductivity (EC)	µS/cm	22	27	6–70	106	Log
pH	–	6.10	6.05	4.82–7.02	106	None
Calcium (Ca)	mg Ca/L	1.21	1.56	0.15–5.47	106	Log
Liming	Yes, Indirectly, Previously, No	–	–	–	106	None
CO ₂	mg C/L	0.71	0.78	0.05–2.00	106	Log
Dissolved Inorganic Carbon (DIC)	mg C/L	1.00	1.23	0.36–4.60	106	Log
Total Organic Carbon (TOC)	mg C/L	5.20	5.46	0.53–16.40	106	Log
Dissolved Inorganic Nitrogen (DIN = NH ₄ + NO ₃)	µg N/L	48	76	1.5–501	106	Log
Total Nitrogen (TN)	µg N/L	310	309	86–695	106	Log
Soluble Reactive Phosphorus (PO ₄)	µg P/L	0.5	0.9	0.5–5	106	Log
Total Phosphorus (TP)	µg P/L	5.0	5.7	1.0–17.0	106	Log
Sediment DIN (sedDIN)	µg N/L sediment	1760	2290	11–11,200	88	Log
Sediment SRP (sedSRP)	µg P/L sediment	8	11	0.01–53.43	88	Log
Sediment organic content (sedOM)	%	8	17	0.3–78.3	88	None

2.3. Lake environmental conditions in southern Norway

We determined the environmental conditions where *Juncus bulbosus* grew, including the sediment (88 lakes) and water chemistry (112 lakes) – see Table 1. County governors assisted with information on liming status of all the lakes.

Water samples were collected at approximately 10 cm depth within the dominant type of *J. bulbosus* growth form where the plant sample was collected. Water for analysis of CO₂ and dissolved inorganic carbon (DIC) were collected in 125 mL gas-tight serum vials with HgCl₂ solution as fixative for CO₂ vials to block biotic uptake and respiration. The samples were stored at room temperature in lake water (in separate plastic containers) until analysis. The remaining analyses were conducted on water sampled in 0.5 L acid-washed plastic bottles, stored in containers with ice packs until analysis.

Lake water chemical parameters were analysed at the Norwegian Institute for Water Research (NIVA) according to Norwegian standards. The pH was analysed on a Metrohm titrator model 799 GPT Titrino (Metrohm AG, Herisau, Switzerland). Conductivity was measured on a Metrohm Conductivity Meter (Metrohm AG, Herisau, Switzerland). Calcium (Ca), nitrate (NO₃) and ammonium (NH₄) were analysed by ion chromatography on a Dionex DX320 (Dionex Corporation, Sunnyvale, California, US). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH₄-N and NO₃-N. Total nitrogen (TotN), total phosphorus (TotP) and phosphate (PO₄) were analysed on a Skalar San Plus auto-analyser (Skalar Analytical B.V., Breda, The Netherlands). The detection limits were 2 µg N L⁻¹ for NH₄, 1 µg N L⁻¹ for NO₃ and 1 µg P L⁻¹ for PO₄ concentrations. Values below the detection limits were given the value of half the detection limits for statistical analyses. Total organic carbon

(TOC), dissolved inorganic carbon (DIC) and carbon dioxide (CO₂) were analysed on a Dohrmann Phoenix 8000 TOC-TC analyser (Teledyne Tekmar, Mason, Ohio, US).

A sediment core of approximately 7 cm length was taken at the location of the plant sample within the stand of the dominant type of *J. bulbosus* growth form representative of the lake (not possible on too rocky substratum or extremely loose (“fluffy”) sediments). The sediment samples were frozen on dry ice immediately after sampling and kept frozen until the analysis.

Sediment porewater was extracted from the thawed sediments in the lab through centrifugation and analysed for PO₄, NO₃, NH₄, water content and organic content. Porewater NH₄ was analysed using protocol B from Holmes et al. (1999). Porewater NO₃ (+ NO₂) and PO₄ were analysed with a SEAL auto-analyser 3 (SEAL Analytical, Bran Luebbe, Norderstedt, Germany) by the cadmium reduction method and molybdenum blue method, respectively. Sediment nutrient concentrations were standardised by sediment water content and expressed as µg nutrient L⁻¹ sediment (nutrient concentration in pore water × proportion of sediment water content). Dry weight was measured after drying the sediments at 105 °C for 24 h. Organic content was measured as ash free dry weight minus dry weight; ash free dry weight being measured after burning the dried sediment sample in a muffle furnace for 2 h at 450 °C and cooling the sample to room temperature in a desiccator.

2.4. Lake catchment scale descriptors in southern Norway

We derived catchment properties for the 112 lakes surveyed in 2007 (Table 2). The catchment areas were delineated according to the procedures described in Larsen et al. (2011a). Annual average temperature,

Table 2

Catchment scale descriptors for 106 of the 112 Norwegian lakes surveyed in summer 2007.

Catchment scale descriptors	Unit	Median	Mean	Range	Transformation
Lake area (LakeA)	km ²	0.22	0.31	0.04–3.65	None
Lake elevation (LakeE)	m	212	240	0–1216	None
Catchment maximum elevation (elevation)	m	370	414	41–1280	None
Catchment area (area)	km ²	8.1	35.0	0.5–1234	Log
Yearly mean temperature	°C	4.8	4.5	–1.6 to –7.2	None
Mean temperature of the coldest month	°C	–4.9	–5.9	–13.4 to –1.1	None
Mean temperature of the warmest month	°C	16.4	16.6	11.7–20.2	None
Nitrogen deposition (Ndep)	mg/m ² /year	0.85	0.82	0.37–1.09	None
Normalized Difference Vegetation Index (NDVI)	–	138	136	102–149	None
Precipitation (rain)	mm/year	1405	1449	635–2811	None
Runoff	mm/year	1077	1383	230–4439	Log
Slope inclination	°	6.67	7.58	1.32–23.	None
Solar irradiation	W/m ²	869	866	808–904	None
Yearly average of global horizontal UVA irradiation	10 kJ/m ²	18,193	18,102	16,377–19,641	None
Yearly average of global horizontal UVB irradiation	kJ/m ²	15,441	15,654	13,722–18,366	None

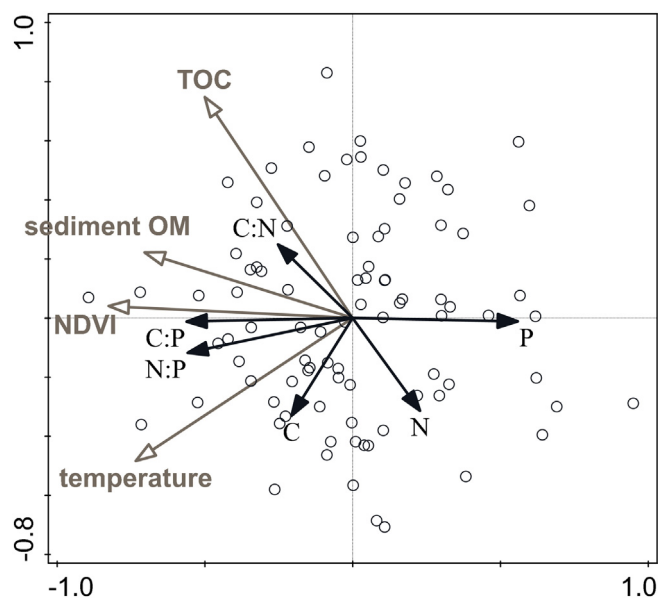


Fig. 2. Redundancy analysis triplot of 88 Norwegian lakes surveyed in summer 2007. Four local and catchment scale explanatory variables (open grey arrows) explained 24% of the variability in C, N, P and C:N:P in *Juncus bulbosus* across 88 sites (open circles). The first two constrained axes explained 18% (axis 1) and 5% (axis 2) of the variability in element content and stoichiometry. See Table S3.

precipitation, runoff and satellite derived normalized difference vegetation index (NDVI, an index describing vegetation cover) as well as data on terrain slope, area types and altitude were obtained according to Larsen et al. (2011b). Atmospheric nitrogen deposition was averaged for each catchment from a digital map of yearly, accumulated total atmospheric nitrogen deposition (including dry deposition) for 1995. The nitrogen deposition map was constructed by spatial interpolation (kriging with a spherical semi-variogram model) on $1^\circ \times 1^\circ$ gridded N deposition output data from the Unified EMEP MSC-W modelling system (<http://www.emep.int/>). Data on solar, UVA and UVB irradiation (based on yearly averages of global horizontal irradiation for the period 1981–1990) were obtained from the photovoltaic geographic information system (PVGIS) of the European Commission Joint Research Centre (JRC) (<http://re.jrc.ec.europa.eu/pvgis/>) – Šúri et al. (2005). Information on hydropower development was obtained from the Norwegian Water Resources and Energy Directorate (NVE).

2.5. Plant tissue C, N, P analyses

All plants were cleaned free of detritus and periphyton by hand. The plants were then freeze-dried in 2006 (Kaste et al., 2007) or air dried at room temperature in this study (and stored over three months in an envelope). The dried plants were cut in pieces and inserted in 2.0 mL vials (Nunc Storage vials, Nalge Nunc International, New York, US) with five

2.8 mm stainless steel beads. The plant material was ground for 45 s at 6500 rpm on a Precellys 24 (Bertin Technologies, Montigny, France) before analysis for C, N and P.

Plant C and N tissue content were analysed on an element analyser (Flash EA 1112 NC Analyser, ThermoFisher Scientific, MA, US). Plant P tissue was determined by acid persulfate digestion followed by the molybdenum blue method (based on Menzel and Corwin, 1965).

2.6. Comparative approach: nutrient gradient data and the homeostatic parameter

We compared *Juncus bulbosus* stoichiometry with other studies on aquatic vascular plants, including hydrophytes and helophytes. We selected a few aquatic ecosystem studies spanning a wide range of external nutrient concentrations for which we had the raw data (Demars and Edwards, 2007b) or we were able to extract the data from published figures with Plot Digitizer version 2.6.3 (Huwaldt, 2013). We also compiled a table of summary data from a wider range of studies for which the raw data were not available.

The regression slope between log change in plant tissue nutrient relative to log change in external nutrient concentrations has been used to characterise the plant's degree of stoichiometric homeostasis and termed the homeostasis parameter $1/H$ (Persson et al., 2010), based on the former homeostasis coefficient H (Sterner and Elser, 2002). The organisms can be arbitrarily classified as homeostatic ($1/H < 0.25$), weakly homeostatic ($0.25 < 1/H < 0.5$), weakly plastic ($0.5 < 1/H < 0.75$) and plastic ($1/H > 0.75$). We calculated $1/H$ for individual species for which we had at least five samples, and communities (all species within a study system).

2.7. Comparative approach: critical nutrient thresholds for maximum yield and growth rate

We compiled a table with as many critical nutrient thresholds as we could find screening papers citing original studies and selected textbooks, encyclopaedia or treatises (1960s onwards). We included both hydrophytes and helophytes since *Juncus bulbosus* can also grow terrestrially and is phylogenetically closely related to other wetland plants from the Juncaceae family, although it was always surveyed in water in the present study. We derived an averaged critical threshold for maximum yield in hydrophytes from which nutrient limitation may be inferred, knowing the uncertainties in nutrient critical threshold across species. The average nutrient critical threshold for maximum growth rate was taken as $1.6 \times$ averaged critical threshold for maximum yield following Colman et al. (1987).

2.8. Statistics

From the 112 lakes surveyed in 2007, we tested the effects of local environmental conditions and catchment scale descriptors on C, N, P, C:N, C:P and N:P of *J. bulbosus* altogether using redundancy analyses

Table 3
Mean carbon, nitrogen and phosphorus content (% dry weight) and element ratios in shoots of aquatic plants in freshwater aquatic ecosystems. The data were ranked by the C:N:P ratios.

Primary producers	C	N	P	C:N:P ^a	Reference
Aquatic macrophytes	37	2.6	0.33	290:17:1	Xia et al., 2014
Submerged vascular plants	43	3.0	0.26	427:26:1	Demars and Edwards, 2007b
<i>J. bulbosus</i>	44	2.4	0.22	516:24:1	Demars and Edwards, 2007b
Submerged vascular plants	41	1.8	0.15	706:27:1	Fernández-Aláez et al., 1999
<i>J. bulbosus</i>	46	1.7	0.16	738:23:1	This study (112 lakes)
Aquatic macrophytes	40	2.1	0.14	738:33:1	Gong et al., 2018
Emergent vascular plants	45	2.1	0.15	775:31:1	Demars and Edwards, 2007b
Emergent vascular plants	43	1.4	0.14	793:22:1	Fernández-Aláez et al., 1999
<i>J. bulbosus</i>	48	2.6	0.13	927:44:1	Rørslett, 1988
Aquatic bryophytes	41	1.8	0.10	1059:40:1	Demars and Edwards, 2007b

^a The average C:N:P presented here was calculated from the average C, N, P values presented in the table, rather than from individual samples or species. This explains possible differences with published averaged C:N:P values (not reported in all studies).

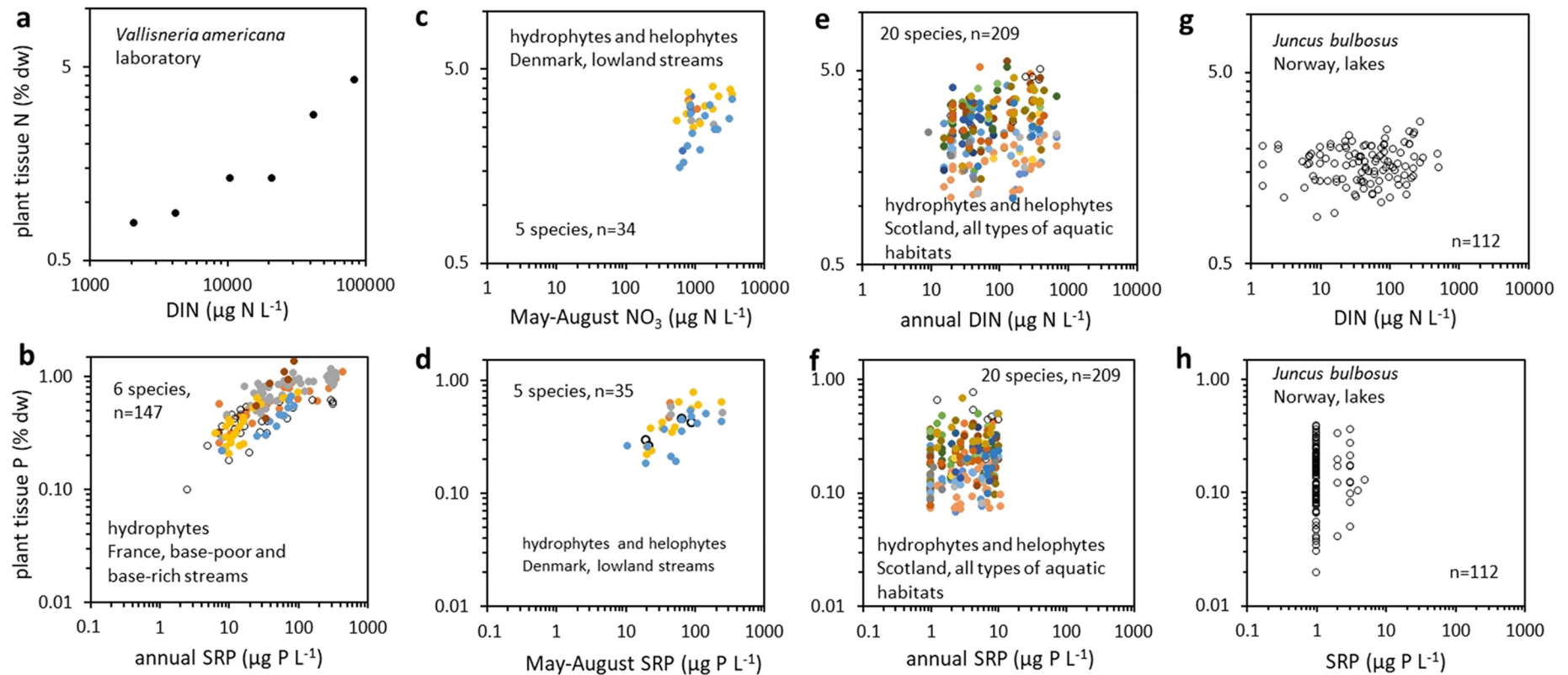


Fig. 3. Aquatic vascular plant tissue nutrient (N, P) response to external nutrient concentrations in increasingly impoverished systems (see also Table 4): (a) one species in the laboratory (Gerloff and Krombholz, 1966), (b) hydrophyte species in stream ecosystems in north-east France (Robach et al., 1996; Thiébaud, 2005), (c, d) hydrophytes and helophytes from Danish lowland streams (Kern-Hansen and Dawson, 1978), (e–f) hydrophytes and helophytes across aquatic habitats in Scotland (Demars and Edwards, 2007b), (g, h) one species in lakes and rivers in southern Norway (this study, all summer data). SRP values below the detection limit ($1 \mu\text{g P L}^{-1}$) were plotted at $1 \mu\text{g P L}^{-1}$. The symbols with different colours represent different species within a study system. DIN = dissolve inorganic nitrogen; SRP = soluble reactive phosphorus.

(RDA), with forward stepwise selection of predictor variables using 9999 Monte Carlo random permutations, computed with Canoco 5.0 (ter Braak and Šmilauer, 2012). Since the selection of variables is dependent on the order of selection, we also checked alternative explanatory variables with similar predictive power. The response variables and some of the predictive variables were log transformed to normalise the data prior to statistical analyses (Tables 1 and 2). We then used multiple linear regressions with model selection using the “step” function (Akaike information criterion) of the “mass” package in R (Venables and Ripley, 2002) to test the effect of environmental variables selected by the RDAs on C, N, P tissue content. Missing data reduced the number of lakes to 106 lakes, including a subset of 88 lakes with sediment data.

3. Results and discussion

3.1. CNP in *Juncus bulbosus* and relation to external environmental conditions

The average ($\pm 95\%$ confidence interval) nutrient content in *J. bulbosus* from the 112 lakes were C = $46(\pm 0.3)$, N = $1.7(\pm 0.07)$, P = $0.16(\pm 0.02)$ % of plant tissue dry weight. The medians were not different to the averages. The inter-lake coefficients of variation were 4, 22 and 53%, respectively, nearly twice as much as intra-lake variability for

N and P (see method). The average ($\pm 95\%$ confidence interval) molar ratios were C:P = 1072 ± 159 , C:N = 33 ± 1 and N:P = 31 ± 3 . The medians were C:P = 787, C:N = 32, N:P = 27, respectively, and the coefficients of variation were 80, 24, 59%, respectively. There were no correlations between the C and N or P contents, but a positive correlation between N and P ($r = 0.59$, $P = 1 \times 10^{-11}$, log transformed data).

The redundancy analyses from the subset 88 lakes for which we had sediment data revealed four main variables related to *Juncus bulbosus* element content and stoichiometry: NDVI (catchment vegetation cover), sediment organic matter, temperature, total organic carbon (which also correlated strongly with light attenuation) – see Fig. 2, Supplementary Table S1. Together these variables explained 24% of the variance in element content and stoichiometry ($\text{adj}R^2 = 0.21$, pseudo-F = 6.6, $P \leq 0.0001$). The same set of explanatory variables (excluding sediment data) was selected for the 106 lakes (Supplementary Table S2). Overall, P tissue content and the related C:P and N:P formed the strongest response to the external environmental variables.

The multiple regression based on 88 lakes showed that C was positively related to temperature and negatively with catchment area ($\text{adj}R^2 = 0.29$, $F = 18$, $P = 2 \times 10^{-7}$), N was negatively related to total organic carbon ($\text{adj}R^2 = 0.13$, $F = 14$, $P = 0.0003$), and P was negatively related to sediment organic matter and NDVI ($\text{adj}R^2 = 0.29$, $F = 19$, $P = 2 \times 10^{-7}$).

Table 4
Plasticity in plant tissue nitrogen and phosphorus relative to external DIN and SRP (total dissolved P in Scotland) concentrations in the water column. n = number of samples, type = submerged (S) versus emergent/floating leaved (E), 1/H homeostasis parameter (regression slope). NA = not applicable for lack of data.

	n	Type	Nitrogen			Phosphorus		
			1/H	R ²	P value	1/H	R ²	P value
Laboratory								
<i>Vallisneria americana</i>	6	S	0.46	0.91	0.003	NA	NA	NA
Streams, France								
<i>Callitriche platycarpa</i>	12	S	NA	NA	NA	0.47	0.84	2.9E-05
<i>Ranunculus peltatus</i>	6	S	NA	NA	NA	0.78	0.74	0.028
<i>Callitriche obtusangula</i>	32	S	NA	NA	NA	0.27	0.58	3.6E-07
<i>Elodea canadensis</i>	25	S	NA	NA	NA	0.26	0.68	3.6E-07
<i>Elodea nuttallii</i>	49	S	NA	NA	NA	0.21	0.61	4.4E-11
<i>Berula erecta</i>	23	S	NA	NA	NA	0.42	0.66	2.8E-06
Community (6 species)	147	S	NA	NA	NA	0.31	0.6	5.3E-31
Streams, Denmark								
<i>Ranunculus peltatus</i>	12/13	S	0.17	0.37	0.037	0.45	0.72	0.00025
<i>Sparganium emersum</i>	13	E	0.28	0.36	0.031	0.30	0.46	0.011
Community ^a (5 species)	34/35	S + E	0.20	0.43	0.011	0.32	0.43	0.00002
Aquatic ecosystems, Scotland								
<i>Callitriche hamulata</i>	8	S	−0.12	0.08	0.50	0.5	0.12	0.40
<i>Elodea canadensis</i>	9	S	0.14	0.23	0.19	0.03	0.00	0.95
<i>Juncus bulbosus</i>	11	S	0.07	0.07	0.42	0.52	0.40	0.037
<i>Littorella uniflora</i>	11	S	0.07	0.04	0.57	0.05	0.002	0.91
<i>Lobelia dortmanna</i>	5	S	0.19	0.45	0.21	1.40	0.79	0.042
<i>Myriophyllum alterniflorum</i>	13	S	0.17	0.36	0.031	0.23	0.06	0.41
<i>Potamogeton natans</i>	18	S	0.07	0.08	0.25	0.21	0.03	0.46
<i>Potamogeton obtusifolius</i>	6	S	0.13	0.84	0.010	0.04	0.02	0.80
<i>Potamogeton polygonifolius</i>	12	S	0.15	0.33	0.050	0.09	0.05	0.50
<i>Carex aquatilis</i>	6	E	0.16	0.36	0.21	0.48	0.12	0.50
<i>Carex rostrata</i>	29	E	0.08	0.21	0.012	0.31	0.18	0.024
<i>Carex vesicaria</i>	5	E	0.12	0.31	0.33	0.05	0.01	0.87
<i>Eleocharis palustris</i>	6	E	−0.11	0.14	0.46	0.28	0.21	0.36
<i>Equisetum fluviale</i>	12	E	0.05	0.28	0.078	0.17	0.09	0.36
<i>Hippuris vulgaris</i>	5	E	0.04	0.04	0.74	0.04	0.005	0.91
<i>Menyanthes trifoliata</i>	10	E	−0.2	0.19	0.20	0.32	0.10	0.37
<i>Nymphaea alba</i>	15	E	0.05	0.09	0.26	0.49	0.26	0.051
<i>Ranunculus flammula</i>	6	E	0.01	0.001	0.95	0.54	0.63	0.059
<i>Sparganium angustifolium</i>	13	E	0.14	0.24	0.091	0.09	0.01	0.73
<i>Sparganium erectum</i>	9	E	−0.06	0.02	0.71	−0.05	0.01	0.83
S community (9 species)	93	S	0.14	0.23	1.4E-06	0.35	0.13	0.00036
E community (11 species)	116	E	0.04	0.02	0.18	0.29	0.08	0.0021
S + E community (20 species)	209	S + E	0.07	0.05	0.0011	0.20	0.04	0.0029
Lakes, Norway								
<i>Juncus bulbosus</i>	112	S	0.02	0.01	0.21	0.02	9.4E-05	0.92

^a including *Potamogeton natans* (n = 4), *Veronica anagallis-aquatica* (n = 2), *Elodea canadensis* (n = 3).

The external environmental variables identified to explain plant tissue element content and stoichiometry in *Juncus bulbosus* are likely related to what drives (indirectly) nutrient limitation. The negative relationship between P tissue content and NDVI may reflect a better terrestrial retention of P, limiting P supply to aquatic ecosystems. The lower redox potential of organic sediment may trigger extensive precipitation of oxidised Fe on the root surfaces of *Juncus bulbosus* adsorbing P and preventing its uptake from the sediment porewater into the roots, as for other isoetids (e.g. Christensen and Sand-Jensen, 1998). This could explain the negative relationship between sediment organic matter and P tissue content. TOC affects light availability which in turn could affect N tissue content, an important element for Rubisco, the enzyme fixing CO₂.

3.2. Comparative method: global synthesis

The average carbon content of *Juncus bulbosus* is relatively high for an aquatic plant, although there were little differences between studies, apart from the nutrient rich area of eastern China which had much lower C content (Xia et al., 2014) – Table 3. The average N and P content of *Juncus bulbosus* from the 112 south Norwegian lakes were lower than hydrophytes and *Juncus bulbosus* from Scotland (Demars and Edwards, 2007b), but comparable to another study with extremely low concentrations of soluble reactive P in Spanish lakes (Fernández-Aláez et al., 1999). There were no evidence that the average C:P was closer to emergent than submerged plants.

3.3. Comparative method: external nutrient gradients and plant tissue content

We present in Fig. 3 a synthesis of past studies selected to represent the widest possible nutrient gradients in nitrogen and phosphorus external concentrations. The nutrient concentrations were not always strictly comparable between studies (summer versus annual mean nutrient concentration, NO₃-N versus dissolved inorganic nitrogen), but nonetheless it is clear that across studies the gradient in external nutrient concentration (three-four orders of magnitude) can affect plant N and P content by about one-two orders of magnitude.

The community homeostasis parameter was similar across studies, homeostatic for N ($1/H < 0.25$) and weakly homeostatic for P ($1/H \approx 0.3$). Individual species responses were also either homeostatic or weakly homeostatic, with many individual species showing no significant relationships (Table 4). The maximum observed values for individual species with at least 10 samples along a nutrient gradient were $1/H \approx 0.5$. The best regressions between plant tissue nutrient content and external nutrient concentrations were from systems rich in inorganic N with a strong gradient in SRP (e.g. Kern-Hansen and Dawson, 1978; Robach et al., 1996; Thiébaud, 2005) – Fig. 3b–d. The lack of significant relationships for individual species in the Scottish and Norwegian studies may simply be due to a lack of P gradient (especially Norway) and P limitation also preventing the increase in plant tissue N as seen in the laboratory assays in the absence of P limitation (Fig. 3a), due to fairly homeostatic N:P molar ratios in plant tissues (Demars and Edwards, 2007b, this study).

3.4. Comparative method: evidence of nutrient limitation in plant tissue

The presence of nutrient limitation may be identified with the plant tissue nutrient critical threshold for maximum growth rate or yield, as proposed by Gerloff and Krombholz (1966). We summarised our compilation of nutrient critical threshold in Table 5. While we acknowledged the variability between methods and species, we calculated averaged N, P and N:P critical thresholds from the seven species for which we had both N and P, under non-limiting inorganic carbon supply. The seven species had different growth forms and included one marine species. The uncertainties were represented by the standard error

of the mean. We then produced a plot of P against N tissue concentrations on log axes to visualise the N, P and N:P nutrient thresholds and their uncertainties (Fig. 4). We compared the Scottish data, including submerged species, emergent species and *Juncus bulbosus* (Demars and Edwards, 2007b) to *Juncus bulbosus* surveyed in 112 lakes in southern Norway. The N and P of *Juncus bulbosus* was very variable (as expected from our survey design), with clear evidence of N (9%), P (25%) and co-limitation (20%) for maximum yield (Fig. 4a). Most of the shoots appeared nutrient co-limited (79%) for maximum growth rate, with only 15% and 2% strictly limited by N and P, respectively (Fig. 4b). Note that the nutrient thresholds were derived under laboratory conditions with high pCO₂ and enough essential nutrients, so it is likely that the apparent nutrient thresholds may be higher under more stressful field conditions (e.g. lower pCO₂, Titus and Andorfer, 1996, Madsen et al., 1998), at least in low alkalinity aquatic ecosystems (cf. Hussner et al., 2019).

Table 5

Aquatic plant tissue critical nutrient thresholds (% dry weight) under which yield or growth rate were limited by nutrient availability. The critical molar N:P ratio represents the optimum balance of nutrient for maximum yield or growth rate.

Species	N	P	N:P	Ref.	Comments
Hydrophytes					
<i>Vallisneria americana</i> ^a	1.30	0.13	22.1	[1]	Max. yield
<i>Elodea canadensis</i>	2.24	n.d.		[11]	Max. RGR
<i>Elodea nuttallii</i>	1.60	0.14	25.3	[2]	95% max. yield
	2.80	n.d.		[12]	Max. RGR, low CO ₂
	1.54	n.d.		[12]	Max. RGR
<i>Ceratophyllum demersum</i>	1.30	0.10	28.8	[2]	95% max. yield
<i>Myriophyllum spicatum</i> ^b	0.75	0.07	23.7	[2] ^e	95% max. yield
	n.d.	0.29–0.32		[5] ^f	90% max. photosynthesis
	n.d.	0.37		[5,8] ^f	95% max. photosynthesis
	n.d.	0.21–0.23		[8] ^g	90% max. yield
	n.d.	0.25–0.27		[8] ^g	95% max. yield
<i>Myriophyllum aquaticum</i>	1.35	0.16	18.7	[14]	95% max. yield
<i>Lemna minor</i> ^a	0.90	0.08	24.9	[2]	95% max. yield
<i>Callitriche cophocarpa</i>	1.82	n.d.		[12]	Max. RGR
<i>Ruppia maritima</i>	2.5–3.0	0.25–0.35	20.3	[7]	Max. yield
Helophytes					
<i>Typha latifolia</i> ^c	1.22	0.25	10.8	[3]	Max. yield
<i>Bolboschoenus maritimus</i> ^c	1.94	0.40	10.7	[3]	Max. yield
<i>Acorus calamus</i> ^c	2.73	0.60	10.1	[3]	Max. yield
<i>Oryza sativa</i> ^d	2.40	0.12–0.18	35.4	[4,6]	Max. yield
Wetland plant communities	1.3–2.0	0.07–0.10	10–20	[15]	Max yield
Bryophytes ^a					
<i>Sphagnum</i> spp.	1.20	n.d.		[13,14]	Growth limitation
<i>Sphagnum balticum</i>	1.10	n.d.		[9]	Productivity limitation
<i>Sphagnum magellanicum</i>	n.d.	0.10		[9]	Productivity limitation
<i>Warnstorffia fluitans</i>	n.d.	0.08		[16]	Max. RGR

RGR = relative growth rate; max. = maximum; n.d. = not determined; ref. = references: [1] Gerloff and Krombholz (1966); [2] Gerloff (1975); [3] Dykijová (1978); [4] Angladette in Nelson (1980); [5] Schmitt and Adams (1981); [6] Mikkelsen & Hunziker in Olson and Kurtz (1982); [7] Thursby (1984); [8] Colman et al. (1987); [9] Aerts et al. (1992); [10] Sytsma and Anderson (1993); [11] Madsen and Baattrup-Pedersen (1995); [12] Madsen et al. (1998); [13] Lamers et al. (2000); [14] Bragazza et al. (2004); [15] Güsewell, 2004; [16] Riis et al. (2010).

^a Whole shoot.

^b Same clone.

^c Above-ground part.

^d Young leaves at maximum tillering for N and onset of flowering for P.

^e 2nd one inch index segments.

^f 10-cm growth tips.

^g 2.5-cm growth tips.

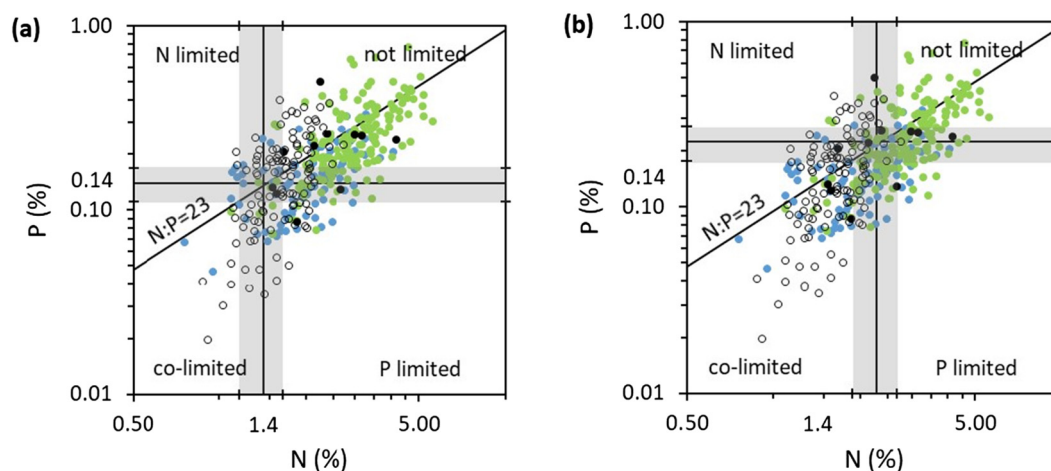


Fig. 4. Variability in N and P plant tissue nutrient content (% dry weight) against average (\pm standard error of the mean, grey shade) nutrient N (1.4 ± 0.25), P (0.14 ± 0.03) and N:P (23.4 ± 3.3) critical thresholds for (a) maximum yield and (b) maximum growth rate determined for hydrophytes from a literature review of laboratory assays (see Methods and Table 5). Tissue nutrient content from shoots of *Juncus bulbosus* (open symbols, 112 lakes, Norway) overlaid on hydrophytes (green), helophytes (blue) and *Juncus bulbosus* (black) values from across aquatic habitats in Scotland (Demars and Edwards, 2007b).

4. Conclusions

Phosphorus tissue content in *Juncus bulbosus* (and its related C:P and N:P stoichiometric ratios) formed the strongest response to the external environmental variables and P was negatively related to sediment organic matter (Fe root plaque limiting P uptake) and NDVI (less P leaching to lakes). The general lack of direct response to external nutrient gradient may be simply due to a lack of P gradient (especially in Norway) preventing the increase in plant tissue N (despite a strong DIN gradient) due to strict plant tissue N:P molar ratios, and possibly explaining the large amount of nutrient co-limitation for maximum growth rate.

Our comparative approach showed that the nutrient content and stoichiometry of *Juncus bulbosus* was similar to other submerged aquatic plants growing in nutrient poor aquatic ecosystems, contrary to previous thoughts (e.g. Rørslett, 1988). In southern Norway, the mass development of *Juncus bulbosus* may be primarily triggered by changes in P availability, rather than CO_2 or inorganic N, as previously thought (Schneider et al., 2013; Lucassen et al., 2016), although co-limitations are also possible. We speculate that the mass development of *Juncus bulbosus* in phosphorus poor ecosystems could be an early indicator of increasing P fluxes through these ecosystems which are less limited by N due to atmospheric N deposition.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2019.04.366>.

Data availability

The compilation of a global database to support our comparative approach can help interpreting local data, and we make our data freely available to encourage its use and development. Table S3 gives the plant tissue nutrient content of *Juncus bulbosus* for the 112 Norwegian lakes, together with all the environmental variables as described in Tables 1 and 2. Table S4 provides the Scottish plant tissue data with geographical coordinate according to British Grid Reference OSGB 1936 and water column nutrient data (in $\mu\text{g N}$ or P L^{-1}). The Scottish data is a subset of previous publications (Demars and Edwards, 2007b, 2007a, 2008).

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