**Article**

**Juncus Bulbosus** Tissue Nutrient Concentrations and Stoichiometry in Oligotrophic Ecosystems: Variability with Seasons, Growth Forms, Organs and Habitats

Therese F. Moe 1, Dag O. Hessen 2 and Benoît O. L. Demars 1,*

1 Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, 0349 Oslo, Norway; therese.fosho.moe@niva.no
2 Department of Biosciences, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway; d.o.hessen@mn.uio.no
* Correspondence: benoit.demars@niva.no

**Abstract:** Aquatic plant nutrient concentrations provide important information to characterise their role in nutrient retention and turnover in aquatic ecosystems. While large standing biomass of aquatic plants is typically found in nutrient-rich localities, it may also occur in oligotrophic ecosystems. *Juncus bulbosus* is able to form massive stands even in very nutrient-dilute waters. Here we show that this may be achieved by tissues with very high carbon-to-nutrient ratios combined with perennial (slow) growth and a poor food source for grazers inferred from plant stoichiometry and tissue nutrient thresholds. We also show that the C, N, P and C:N:P stoichiometric ratios of *Juncus bulbosus* vary with the time of year, habitats (lakes versus rivers) and organs (roots versus shoots). We found no differences between growth forms (notably in P, inferred as the most limiting nutrient) corresponding to small and large plant stands. The mass development of *J. bulbosus* requires C, N and P, whatever the ecosystem (lake or river), and not just CO2 and NH4, as suggested in previous studies. Since macrophytes inhabiting oligotrophic aquatic ecosystems are dominated by isoetids (perennial plants with a high root/shoot ratio), attention should be paid to quantifying the role of roots in aquatic plant stoichiometry, nutrient turnover and nutrient retention.

**Keywords:** plant stoichiometry; carbon; nitrogen; phosphorus; river; lake; nutrient

1. **Introduction**

Aquatic plants tend to have higher N and P and lower C:N and C:P ratios than terrestrial plants [1,2]. This generally corresponds to higher growth rates and decomposition rates, as well as higher herbivory [1–3]. Standing biomasses of freshwater macrophyte meadows are equivalent to those of grassland ecosystems [1]; hence, aquatic plants can actively contribute to nutrient cycling [4–6], as well as through the uptake, retention and release of nutrients from the sediment to the water column via decomposition and herbivory [7,8].

The role of aquatic plants in nutrient net retention may be very modest relative to external loading in nutrient-rich ecosystems. For example, studies on British calcareous lowland rivers impacted by agriculture and point-source effluents, bearing high standing biomass [9], reported very small plant nutrient retention relative to total flux: less than 1 to 2.5% for P [10–12] and 0.2 to 2% for N [10,11]. Slightly higher retention rates by aquatic plants (up to 10–13% of the dissolved inorganic N river flux during the summer) were reported in a lowland river in the Netherlands [13]. Large standing biomass of aquatic plants is also known from oligotrophic aquatic ecosystems [14–16], where aquatic plant nutrient retention may be more significant [7,16].

Aquatic plant nutrient retention is often determined for the shoot without the roots, but in nutrient-poor aquatic ecosystems, aquatic plants tend to be dominated by isoetids.
characterised by high root/shoot ratios [17]. There were more differences between roots and shoots for N than P concentrations in *Lobelia dortmanna*, especially during the summer, with larger N concentrations in shoots than roots [18]. Relative differences in N and P between roots and shoots can be driven by light and nutrient availability [19–21]. In northwest Europe, few aquatic plant species grow to form large submerged stands (1–3 m tall) in oligotrophic ecosystems, and among those species, *Juncus bulbosus* is the only (facultative) perennial type, also playing an important role in ecosystem structure and functions [22]. Since *J. bulbosus* is also widely distributed in northern Europe [23], it makes it an important species to study.

In southern Norway, the mass development of *Juncus bulbosus* occurs in lakes and rivers (with up to 500–1100 g dry mass m$^{-2}$ [24,25]), despite extremely low nutrient concentrations [26,27]. *J. bulbosus* can have a high root/shoot ratio in flowing water (1.5 ± 0.5, based on dry mass m$^{-2}$, Demars, unpublished), and so root nutrient retention should not be overlooked [28]. A detailed study on Norwegian lakes identified several local- and catchment-scale drivers of *J. bulbosus* tissue stoichiometry [26]. Some patterns in the tissue C, N, P concentrations and C:N:P stoichiometry of *J. bulbosus* in Norwegian lakes and rivers were identified [27] using averaged plant data from different years and analysed in different ways (entire plant versus shoots). Thus, it remains to directly test for more specific sources of variability such as seasons, growth forms, organs and habitats.

We aimed to characterise *Juncus bulbosus* tissue nutrient concentrations and stoichiometry in southern Norway, an important step towards characterising the potential role of macrophytes in nutrient retention in oligotrophic aquatic ecosystems. Here we set out to (i) test whether *Juncus bulbosus* C, N, P and C:N:P in lakes can be related to growth forms (abundance) and the time of year; (ii) test for differences in *Juncus bulbosus* elemental concentrations and stoichiometry between habitats (lakes versus rivers), plant organs (roots versus shoots) and habitats × plant organs; and (iii) assess the likelihood of nutrient limitation for yield (standing biomass) using a comparative approach [26].

2. Results

2.1. Growth Forms and the Time of Year

C, N, P (46 ± 2, 1.7 ± 0.2, 0.16 ± 0.08%) and C:N:P (1073:31:1) in *Juncus bulbosus* varied greatly and significantly between the six lakes surveyed in 2006. There were, however, no significant differences in C, N, P and C:N:P between large growth forms and rosette leaves or new shoots. The effect of growth form on N concentrations in plant tissue (close to significance after correction for multiple testing) explained 9.3% of the variance, whereas the full mixed-effects model (including random effects) explained 34% of the variance. This was mostly due to differences (median) in N tissue concentrations in the large growth form (June: 2.2%, October: 2.6%) versus rosette (June: 2.6%, October: 2.8%). The effect of the time of sampling (June versus October) on P concentrations in plant tissue explained 14% of the variance, and the full model explained 49% of the variance. The median P concentration was lower in October (0.09%) than in June (0.13%), and correspondingly, the C:P and N:P were higher: C:P = 1332 versus 954; N:P = 61 versus 45, respectively (Table 1).

Table 1. Mixed-effects model to test the effects of the time of sampling (spring versus autumn) and growth forms (rosette, large growth form, new shoots) in *Juncus bulbosus*. Data from 2006 (see Methods). Bonferroni correction for multiple testing $\alpha = 0.05/12 = 0.004$.

| Time of Sampling | Growth Form |
|------------------|-------------|
|                  | C           | N           | P           | C:N           | N:P          | P          |
|                  | $\chi^2$    | $P$         | $\chi^2$   | $P$          | $\chi^2$    | $P$        |
| C                | 0.2         | 0.68        | 1.8        | 1.41         |             |            |
| N                | 2.3         | 0.13        | 9.9        | 0.007        |             |            |
| P                | 20.1        | 7 $\times$ 10$^{-6}$ | 1.5        | 0.47         |             |            |
| C:N              | 1.9         | 0.17        | 8.1        | 0.02         |             |            |
| C:P              | 20.2        | 7 $\times$ 10$^{-6}$ | 1.2        | 0.55         |             |            |
| N:P              | 33.1        | 9 $\times$ 10$^{-9}$ | 7.2        | 0.03         |             |            |
C, N, P (46 ± 2, 1.7 ± 0.3, 0.18 ± 0.09%) and C:N:P (843:26:1) in *Juncus bulbosus* were also very variable in the ten lakes surveyed in summer 2008. Neither growth form nor the time of sampling (June *versus* September) could explain the variability in C, N, P or C:N:P (Table 2).

**Table 2.** Mixed-effects model to test the effects of the time of sampling (early *versus* late summer) and growth forms (rosette, large growth form, new shoots) in *Juncus bulbosus*. Data from 2008 (see Methods). Bonferroni correction for multiple testing $\alpha = 0.05/12 = 0.004$.

| Time of Sampling | Growth Form |
|------------------|-------------|
| $\chi^2_1$ | $P$ | $\chi^2_1$ | $P$ |
| C | 0.03 | 0.87 | 2.1 | 0.15 |
| N | 1.1 | 0.29 | 0.7 | 0.41 |
| P | 0.1 | 0.77 | 0.7 | 0.39 |
| C:N | 1.1 | 0.30 | 1.7 | 0.20 |
| C:P | 0.1 | 0.78 | 0.4 | 0.51 |
| N:P | 0.4 | 0.51 | 1.3 | 0.25 |

2.2. **Plant Organs (Roots *versus* Shoots) and Habitats (Lakes *versus* Rivers)**

We first checked for differences in environmental conditions. The 16 lakes and 28 river sites sampled for this purpose differed in this context. The lakes had higher total N concentrations (median: 300 *versus* 230 µg N L$^{-1}$, $F = 19$, $P = 4 \times 10^{-5}$), a higher sediment organic matter content (18 *versus* 2%, $F = 22$, $P = 1 \times 10^{-5}$), a higher proportion of sediment pore water (76 *versus* 36%, $F = 28$, $P = 1 \times 10^{-6}$) and higher electric conductivity (32 *versus* 16 µS cm$^{-1}$, $F = 41$, $P = 1 \times 10^{-10}$). There were no differences in inorganic N or P concentrations either from the sediment or the water column between lakes and rivers.

C tissue concentration was similar across organs and habitats (Figure 1). The roots had a lower N concentration ($F = 86$, $P = 4 \times 10^{-14}$) and lower P concentration ($F = 13$, $P = 0.0006$) than the shoots. N tissue concentration was higher in the rivers than the lakes ($F = 20$, $P = 2 \times 10^{-5}$) and similarly for P, although not statistically significant. C:N and C:P reflected N and P, but N:P was similar across organs and habitats. There were no significant interactions between organs and habitats. The average shoot concentrations were 1.76 *versus* 2.40% for N and 0.12 *versus* 0.16% for P in lakes *versus* rivers, respectively. The average shoot C:N:P ratios were 1091:35:1 *versus* 824:38:1 in lakes *versus* rivers, respectively. Multivariate analyses identified organs (shoot *versus* root) as the main factor able to explain the variance in plant tissue concentrations and stoichiometry, together with habitats (lake *versus* river), total P and electric conductivity, independently of the way missing data were handled (Table 3). The forward selection of these significant factors produced a highly significant ($P < 0.001$) model with an adjR$^2$ of 30% (Table 3).

**Table 3.** Percentage of variance in plant nutrient concentrations (C, N, P) and stoichiometry (C:N:P) explained by organs (root *versus* shoot), habitats (lake *versus* river) and environmental variables (water and sediment chemistry) using redundancy analyses: singly and after stepwise selection. Only significant explanatory variables are shown. $n = 80$, missing data were replaced by averages across all samples. TP = total P, EC = electric conductivity.

| Percentage of Explained Variance |
|----------------------------------|
| **Singly** | **P** | **After Stepwise Regression** | **P** | **Selection Order** |
| Organs   | 20.8 | <0.001 | 20.8 | <0.001 | 1 |
| Habitats | 4.6  | 0.004 | 4.1  | 0.028 | 3 |
| TP       | 4.6  | 0.006 | 3.9  | 0.040 | 4 |
| lnEC     | 3.0  | 0.027 | 4.3  | 0.019 | 2 |
Figure 1. Differences in the element concentrations and stoichiometry of *Juncus bulbosus* between organs (roots and shoots) and habitats (lakes and rivers)—summer 2010 (Table 4).

Table 4. *Juncus bulbosus* C, N, P tissue concentrations from southern Norway. *n* = number of samples.

| Datasets                          | Year | Description                                                                 |
|-----------------------------------|------|-----------------------------------------------------------------------------|
| 6 lakes                           | 2006 | Replicate *J. bulbosus* sampling of different growth forms in 6 lakes at two seasons (spring and autumn), *n* = 86 |
| 10 lakes                          | 2008 | Single *J. bulbosus* samples (roots and shoots together), two sampling times (early and late summer) and two growth forms, *n* = 40 |
| 16 lakes and 28 river sites       | 2010 | Single *J. bulbosus* samples, two habitats (lakes and rivers) and two organs (roots versus shoots), *n* = 80 |

3. Discussion

Overall, there were little or no differences in plant element concentrations and stoichiometry between growth forms (2006 and 2008 surveys), similarly to between small and large plant stands [27], likely because the plant growth rate was nutrient limited, and the N:P molar ratio was relatively strict [26]. The possible difference in N concentrations (2006 survey only) between growth forms may be due to unbalanced P limitation leading to N “luxury consumption” (Figure 2a).
Figure 2. Tissue nutrient concentrations (% dry weight) from the shoots of *Juncus bulbosus* in (a) six lakes (*n* = 86) surveyed in spring (circles) and autumn (triangles) 2006 and (b) 16 lakes (circles) and 28 river sites (squares) surveyed in summer 2010. Results plotted against average (±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 maximum yield determined for hydrophytes from a literature review of laboratory assays [26].

There were no temporal differences in C, N, P and C:N:P between samples collected in early versus late summer (2008 survey) but large differences in P, C:P and N:P ratios between samples collected in late spring versus autumn (2006 survey). Such seasonal shifts in plant stoichiometry have been reported before for other species and are known to vary between species and elements [29–32]. In *Lobelia dortmanna* (an isoetid), N accumulation occurred under low light availability and slow growth in the autumn for possible use in the following spring and summer growth spurt [18].

The most imbalanced stoichiometry was from samples collected in autumn when the plants had much higher N relative to P, likely indicative of a period with slow growth, indicated by the nutrient thresholds derived from laboratory bioassays (Figure 2a [18,26]). *Juncus bulbosus* had a lower N tissue concentration in lakes than rivers in southern Norway (2010 survey) despite similar external inorganic nutrient concentrations (water and sediment). This may reflect the higher supply rate of nutrients in rivers compared to lakes, especially the more limiting factor P, with most samples below the critical N:P ratio (Figure 2b). Phosphorus limitation may also be more pronounced in lakes than rivers due to a higher organic matter content in lake than river sediments (18% versus 2%, respectively), potentially triggering root iron plaque formation and preventing P uptake, as for other
Plants 2021, 10, x FOR PEER REVIEW

The higher nutrient concentration in shoots compared to roots can be explained by the high N concentrations abundant in chlorophyll and Rubisco (the enzyme-fixing CO$_2$) in the leaf, as well as the energetic requirements associated with photosynthesis, requiring a high concentration of the P-rich adenosine 5′-triphosphate (ATP) [35].

The average P concentrations in the shoots of *Juncus bulbosus* (0.12% in lakes and 0.16% in rivers) in summer 2010 in Norway were generally lower than *Juncus bulbosus* and associated species recorded in Scotland (*Juncus bulbosus* P = 0.22% and molar C:P = 639—Figure 3 [14]) but comparable to some species in Spanish oligotrophic lakes (e.g., *Potamogeton natans* P = 0.12% and molar C:P = 965; *Littorella uniflora* P = 0.12% and C:P = 861; *Juncus heterophyllus* P = 0.09% and C:P = 1218—Figure 3 [36]). These average concentrations suggested no limitations for maximum yield (critical threshold: P = 0.14%, Figure 3) but limitations for maximum growth rates (critical threshold: P = 0.22% [26]). These low P concentrations and high C:P molar ratios (lake C:P = 1091 and rivers C:P = 824) in plant tissue combined with perennial growth give the opportunity to form mass stands under extremely low nutrient regimes. They also make the plant relatively unattractive to generalist grazers [2,8] in addition to being a cyanogenic plant [37]. In southern Norway, aquatic plants including *Juncus bulbosus* were also found to have higher heavy metal concentrations in their tissue than terrestrial plants but suggested aquatic plants as a source of sodium for moose [38].

![Figure 3. Tissue nutrient concentrations (% dry weight) from (i) the shoots of *Juncus bulbosus* (filled circles) and associated species (open circles) collected in Scotland during the summer across all types of aquatic habitats (n = 102 samples [14]); (ii) 12 species of hydrophytes and helophytes (open squares) from oligotrophic Spanish lakes [36]. Species list from Scotland with the number of samples (corresponding to the number of datapoints per species on the graph): Callitriche hamulata (8), Isoetes lacustris (4), Juncus bulbosus (11), Littorella uniflora (11), Lobelia dortmanna (5), Myriophyllum alterniflorum (13), Potamogeton alpinus (2), P. natans (18), P. polygonifolius (12), Sparganium angustifolium (12), Subularia aquatica (1), Utricularia intermedia (2) Utricularia vulgaris sensu lato (3). Same nutrient threshold as Figure 2.](image)

Reading through the original publications [24,39,40], no factorial experiments were ever carried out to test the independent and interactive effects of CO$_2$, N and P on the
Juncus bulbosus growth rate and yield. The liming of lakes in Norway promoted J. bulbosus mass development through the mineralisation of the organic matter with the production of CO$_2$, NH$_4$ and inorganic P in sediment pore water [24] until the organic matter became less reactive over time [41]. In the presence of sufficient N and P, CO$_2$ can be a limiting factor [39,40,42]. Excess of CO$_2$ availability relative to N and P is expected to increase the C:N and C:P of autotrophs in nutrient-poor freshwater ecosystems [43–45]. General N and/or P limitations have been reported for the phytoplankton of Norwegian lakes [46,47] and inferred through J. bulbosus plant tissue nutrient concentration and stoichiometry [26].

The repeated story that the mass development of J. bulbosus would only occur under high CO$_2$ and sediment pore water NH$_4$ concentrations [24,27,41,48] can only make sense if the plant can access enough P for its growth because aquatic plant tissue C:N:P stoichiometry is rather homeostatic [14,26], whatever the ecosystem, based on principles of ecological stoichiometry. Thus, in Norwegian lakes and rivers, it is likely that CO$_2$, N and/or P could limit or colimit the growth rate of J. bulbosus. Certainly, the role of P should not be disregarded (this study [26]). J. bulbosus has also been able to produce stands with large biomass in (unlimed) oligotrophic ecosystems through perennial growth where physical conditions allow (high light, low water turbulence) (Figure 4 [49]). There were no significant differences in the water column and sediment pore water nutrient concentrations between stands with low and high J. bulbosus biomass in rivers [50], unlike for lakes shortly after liming [24].

![Figure 4.](image-url)

**Figure 4.** Cont.
4. Materials and Methods

4.1. Juncus Bulbosus

*Juncus bulbosus* is a perennial species common in oligotrophic and ultraoligotrophic European lakes and rivers [51,52]. It has caused problems with mass development along the littoral zones of many lakes and rivers since the mid-1980s [53–55]. Although these problems have receded in some areas [41,56], it remains an issue in southern Norway [15,27] (Figure 4). *J. bulbosus* is an amphibious plant morphologically very plastic, ranging from small tufted terrestrial plants to submerged, floating aquatics, often rooting at the nodes and freely fruiting [57]. In this study, all samples were rooted underwater, and thus *J. bulbosus* may be referred to as *J. aquatica*, with the largest shoots reaching the water surface.

4.2. Growth Form and the Time of Year

In a preliminary study, 86 plant samples (3–10 shoots per lake) were separated into rosette leaves, large growth forms (stem or “column” with annual shoots, as illustrated in [15] and Figure 4) and new annual shoots (rosette-bearing segments) representing the vegetative life cycle of *J. bulbosus*, collected in southern Norway from six lakes in spring (31 May–14 June) and autumn (29 September–12 October, 2006) (Figure 4) [58].

We collected an additional set of plant samples (*n* = 40) from ten lakes with two growth forms (rosette and large growth forms, both present in the ten lakes) and two summer sampling times (21–26 June 2008 and 6–10 September 2008) per lake. The data are available in the Supplementary Information.

4.3. Plant Organs (Roots versus Shoots) and Habitats (Lakes versus Rivers)

Sixteen lakes were sampled during 26–30 July 2010 and 28 river sites (50 m stretches from 15 different rivers) during 4–16 August 2010 to analyse the roots and shoots of *J. bulbosus*. Sites were chosen to span a wide range of *J. bulbosus* growth forms and abundances. A single individual (root and shoot) of *J. bulbosus* per site was picked from the dominant type of growth form or most abundant stand, as previously described [26,27]. Thus, rosette growth forms generally corresponded to stands with low biomass, whereas large growth forms corresponded to stands with large biomass.

The concentrations of NH$_4$, NO$_3$, PO$_4$, Ca, dissolved inorganic carbon (DIC), CO$_2$, total organic carbon (TOC), total N and total P, as well as pH and electric conductivity, were determined for the water column. The concentrations of NH$_4$, NO$_3$ and PO$_4$ in sediment pore water, as well as the percentage of organic matter and proportion of pore water in sediments, were also determined. All samples were collected and analysed, as described in previous studies [26,27]. The data are available in the Supplementary Information.
4.4. 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 [58] or air dried at room temperature for the 2008 and 2010 datasets [27]. The dried plants were ground prior to analyses for C, N and P, as previously described [26,27].

4.5. Statistics

In total, we used three sets of data from southern Norway, briefly summarised in Table 4. Two of the datasets (2008, 2010) were partially used in a previous study designed to test different hypotheses, as explained in the Introduction (Section 1) [27]. Here we did not combine those datasets as in [27] as they were not comparable, and we did not use plant nutrient concentrations and stoichiometry as factors to explain plant abundance (corresponding to growth form) as in [27] but as response variables to test for the effects of seasons, growth forms, organs and habitats. We then interpreted those datasets differently to [27] with a comparative approach based on a review of laboratory bioassays, as previously applied to a dataset collected in 2007 [26].

From the lakes surveyed in 2006 and 2008, we tested for differences in C, N, P and C:N:P with mixed-effects models (allowing for unbalanced design) using sampling time or growth form as the fixed effects and site as the random effect. Sampling time or growth form were also used as random effects when testing for growth form or sampling time, respectively. The response variables were log transformed prior to analyses to normalise the data. Differences were considered significant after correcting for multiple testing using a Bonferroni correction ($\alpha = 0.05/n$, with n number of tests). Statistics were computed in R version 3.5.0 [59] using the lme4 package [60]. The fit of the models with significant fixed effects was assessed with the conditional and marginal coefficient of determination ($R^2$) using the R function r.squaredGLMM from the MuMIn package [61,62]. The conditional $R^2$ represents the variance explained by fixed and random factors together, and the marginal $R^2$ represents the variance explained solely by the fixed effects.

From the lakes and rivers surveyed in 2010, we first tested whether there were any statistical differences in environmental variables, notably in nutrient concentrations (water and sediment) using one-way ANOVA in R. We then tested for the effect of habitats (lake and river), organs (root and shoot) and their interaction on C, N, P and stoichiometric molar ratios using two-way ANOVA in R version 3.5.0 [59]. It was not necessary to apply any data transformations prior to statistical analyses in this dataset.

In addition, we used multivariate analyses (redundancy analyses) to test for the single and collective effects of organs (root, shoot), habitats (lakes, rivers) and environmental variables (water and sediment chemistry) on plant nutrient concentrations (C, N, P) and stoichiometry (C:N, C:P, N:P) using the 2010 dataset. We also calculated sediment pore water nutrients per volume of sediment (concentrations $\times$ pore water volume). Missing values for plant P tissue in 10 samples and sediment pore water in 11 samples (out of 80 samples) were handled in two different ways, either by replacing missing data with averages taken across all samples or simply removing the samples with missing data. This led to virtually the same results. Electric conductivity, NH$_4$ and sediment pore water concentrations were ln transformed to normalise the data. The significance of individual predictors was tested by running 999 unrestricted Monte Carlo random permutations. We then use a forward stepwise selection of significant variables to produce the best models. The multivariate analyses were run with Canoco 5.0 (Microcomputer Power: Ithaca, NY, USA) [63].

5. Conclusions

There was no (or very little) difference in plant nutrient concentrations between growth forms but large differences between the time of year, habitats and organs. The very high C:P ratios recorded here suggested Juncus bulbosus is not attractive to grazers and combined with (slow) perennial growth can reach high standing biomass in nutrient-poor
lakes and rivers as observed. In order to quantify nutrient retention of aquatic plants in oligotrophic aquatic ecosystems, it will be important to sample year-round and quantify the contribution of roots and shoots.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2223-7747/10/3/441/s1, Table S1: Lake 2008 CNP data.xlsx, Table S2: River lake 2010 CNP data.xlsx.

**Author Contributions:** Conceptualization, T.F.M., D.O.H., and B.O.L.D.; data analyses, T.F.M. and B.O.L.D.; data curation, T.F.M. and B.O.L.D.; writing—original draft preparation, B.O.L.D.; writing—review and editing, T.F.M., D.O.H., and B.O.L.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** The project was funded by The Norwegian Research Council, “Krypsivprosjektet på Sørlandet,” Norwegian Institute for Water research (NIVA) and the University of Oslo.

**Data Availability Statement:** All data supporting reported results can be found in the Supplementary Information.

**Acknowledgments:** Thanks to Berit Kaasa for running the element analyses, Susi Schneider and other NIVA employees for helping with fieldwork and Øyvind Kaste for the preliminary study. The manuscript was substantially improved by the pertinent comments of two anonymous referees.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

**References**

1. Cebrian, J. Patterns in the fate of production in plant communities. *Am. Nat.* 1999, 154, 449–468. [CrossRef]
2. Bakker, E.S.; Wood, K.A.; Pages, J.F.; Veen, G.F.; Christianen, M.J.A.; Santamaria, L.; Nolet, B.A.; Hilt, S. Herbivory on freshwater and marine macrophytes: A review and perspective. *Aquat. Bot.* 2016, 135, 18–36. [CrossRef]
3. Enriquez, S.; Duarte, C.M.; Sand-Jensen, K. Patterns in decomposition rates among photosynthetic organisms—The importance of detritus C-N-P content. *Oecologia* 1993, 94, 457–471. [CrossRef]
4. Bernt, M.J.; Tank, J.L.; Royer, T.V.; David, M.B. Nutrient uptake in streams draining agricultural catchments of the midwestern United States. *Freshw. Biol.* 2006, 51, 499–509. [CrossRef]
5. Levi, P.S.; Riis, T.; Alnæe, A.B.; Peipoch, M.; Maetzke, K.; Brus, C.; Baatrup-Pedersen, A. Macrophyte complexity controls nutrient uptake in lowland streams. *Ecosystems* 2015, 18, 914–931. [CrossRef]
6. O’Brien, J.M.; Lessard, J.L.; Plew, D.; Graham, S.E.; McIntosh, A.R. Aquatic macrophytes alter metabolism and nutrient cycling in lowland streams. *Ecosystems* 2014, 17, 405–417. [CrossRef]
7. Barko, J.W.; Smart, R.M. Mobilization of sediment phosphorus by submerged freshwater macrophytes. *Freshw. Biol.* 1980, 10, 229–238. [CrossRef]
8. Grutters, B.M.C.; Gross, E.M.; Bakker, E.S. Insect herbivory on native and exotic aquatic plants: Phosphorus and nitrogen drive insect growth and nutrient release. *Hydrobiologia* 2016, 778, 209–220. [CrossRef]
9. Westlake, D.F.; Casey, H.; Dawson, F.H.; Ladle, M.; Mann, R.H.K.; Marker, A.F.H. The chalk-stream ecosystem. In Proceedings of the IBP-UNESCO Symposium on Productivity Problems of Freshwaters, Kazimiera Dolny, Poland, 6–12 May 1970; Kajak, Z., Hillbricht-Ilkowska, A., Eds.; Polish Scientific Publishers: Warsaw, Poland, 1972; pp. 615–635.
10. Ladle, M.; Casey, H. Growth and nutrient relationships of Ranunculus penicillatus var calcareus in a small chalk stream. In *Proceedings of the European Weed Research Council 3rd International Symposium on Aquatic Weeds*; European Weed Research Society: Oxford, UK, 1971; pp. 53–62.
11. Westlake, D.F. The biology of aquatic weeds in relation to their management. In *Proceedings of the 9th British Weed Control Conference*; British Weed Control Council: Brighton, UK, 1968; pp. 371–381.
12. House, W.A.; Duplat, D.; Denison, E.H.; Hernville, P.; Dawson, F.H.; Cooper, D.M.; May, L. The role of macrophytes in the retention of phosphorus in the river Thame, England. *Chem. Ecol.* 2001, 17, 271–291. [CrossRef]
13. Desmet, N.J.S.; Van Belleghem, S.; Seuntjens, P.; Bouma, T.J.; Buis, K.; Meire, P. Quantification of the impact of macrophytes on oxygen dynamics and nitrogen retention in a vegetated lowland river. *Phys. Chem. Earth* 2011, 36, 479–489. [CrossRef]
14. Demars, B.O.L.; Edwards, A.C. Tissue nutrient concentrations in freshwater aquatic macrophytes: High inter-taxon differences and low phenotypic response to nutrient supply. *Freshw. Biol.* 2007, 52, 2073–2086. [CrossRef]
15. Moe, T.F.; Brysting, A.K.; Andersen, T.; Schneider, S.C.; Kaste, O.; Hessen, D.O. Nuisance growth of Juncus bulbosus: The roles of genetics and environmental drivers tested in a large-scale survey. *Freshw. Biol.* 2013, 58, 114–127. [CrossRef]
16. Preiner, S.; Dai, Y.; Pucher, M.; Reitsema, R.E.; Schoelynck, J.; Meire, P.; Hein, T. Effects of macrophytes on ecosystem metabolism and net nutrient uptake in a groundwater fed lowland river. *Sci. Total Environ.* 2020, 721, 137620. [CrossRef]
17. Murphy, K.J.; Rørslett, B.; Springel, I. Strategy analysis of submerged lake macrophyte communities: An international example. *Aquat. Bot.* 1990, 36, 303–323. [CrossRef]

18. Moeller, R.E. Seasonal changes in biomass, tissue chemistry, and net production of the evergreen hydrophyte, *Lobelia dortmanna*. *Can. J. Bot.* 1978, 56, 1425–1433. [CrossRef]

19. Cronin, G.; Lodge, D.M. Effects of light and nutrient availability on the growth allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia* 2003, 137, 32–41. [CrossRef]

20. Guisan, A.; Tingley, R.; Baumgartner, J.B.; Naujokaitis-Lewis, I.; Sutcliffe, P.R.; Tulloch, A.I.T.; Regan, T.J.; Brotons, L.; McDonald-Madden, E.; Mantyka-Pringle, C.; et al. Predicting species distributions for conservation decisions. *Ecol. Lett.* 2013, 16, 1424–1435. [CrossRef]

21. Garbey, C.; Murphy, K.J.; Thiébaut, G.; Muller, S. Variation in P-content in aquatic plant tissues offers an efficient tool for determining plant growth strategies along a resource gradient. *Freshw. Biol.* 2004, 49, 346–356. [CrossRef]

22. Velle, G.; Skoglund, H.; Barlaup, B.T. Effects of nuisance submerged vegetation on the fauna in Norwegian rivers. *Hydrobiologia* 2021. [CrossRef]

23. Hultén, E.; Fries, M. *Atlas of North European Vascular Plants: North of the Tropic of Cancer*; Koeltz: Königstein, Germany, 1986.

24. Roelofs, J.G.M.; Brandrud, T.E.; Smolders, A.J.P. Massive expansion of *Juncus bulbosus* L. after liming of acidified SW Norwegian lakes. *Aquat. Bot.* 1994, 48, 187–202. [CrossRef]

25. Rørslett, B.; Brandrud, T.E.; Johansen, S. Tilgroing i Terskelbasseng i Otra ved Valle. Problemanalyse og Forslag om Tiltak. Report No 2442; Norsk Institutt for Vannforskning: Oslo, Norway, 1990.

26. Moe, T.F.; Hessen, D.O.; Demars, B.O.L. Functional biogeography: Stoichiometry and thresholds for interpreting nutrient limitation in aquatic plants. *Sci. Total Environ.* 2019, 677, 447–455. [CrossRef] [PubMed]

27. Schneider, S.C.; Moe, T.F.; Hessen, D.O.; Kaste, O. Juncus bulbosus nuisance growth in oligotrophic freshwater ecosystems: Different triggers for the same phenomenon in rivers and lakes? *Aquat. Bot.* 2013, 104, 15–24. [CrossRef]

28. Maine, M.A.; Sürhe, N.L.; Panigatti, M.C.; Pizarro, M.J.; Emiliani, F. Relationships between water chemistry and macrophyte chemistry in lotic and lentic environments. *Arch. Für Hydrobiol.* 1999, 145, 129–145. [CrossRef]

29. Baldy, V.; Trémolières, M.; Andrieu, M.; Belliard, J. Changes in phosphorus content of two aquatic macrophytes according to water velocity, trophic status and time period in hardwater streams. *Hydrobiologia* 2007, 575, 343–351. [CrossRef]

30. Thiébaut, G.; Muller, S. Linking phosphorus pools of water, sediment and macrophytes in running waters. *Ann. Limnol. Int. J. Limnol.* 2003, 39, 307–316. [CrossRef]

31. Thiébaut, G. Does competition for phosphate supply explain the invasion pattern of *Elodea* species? *Water Res.* 2005, 39, 3385–3393. [CrossRef]

32. Reitsema, R.E.; Preiner, S.; Meire, P.; Hein, T.; Dai, Y.R.; Schoelynck, J. Environmental control of macrophyte traits and interactions with metabolism and hydromorphology in a groundwater-fed river. *River Res. Appl.* 2021, 37, 294–306. [CrossRef]

33. Christensen, K.K.; Sand-Jensen, K. Precipitated iron and manganese plaques restrict root uptake of phosphorus in *Lobelia dortmanna*. *Aquat. Ecol.* 2008, 413–422. [CrossRef]

34. Sterner, R.W.; Elser, J.J. *Ecological Stoichiometry. The biology of Elements from Molecules to the Biosphere*; Princeton University Press: Princeton, NJ, USA, 2002.

35. Fernández-Aláez, M.; Fernández-Aláez, C.; Bécares, E. Nutrient content in macrophytes in Spanish shallow lakes. *Hydrobiologia* 1999, 408–409, 317–326.

36. Hegnauer, R.; Ruijgrok, H.W. *Lilaea scilloides und Juncus bulbosus zwei neue cyanogene pflanzen*. *Phytochemistry* 1971, 10, 2121–2124. [CrossRef]

37. Ohlson, M.; Staaland, H. Mineral diversity in wild plants: Benefits and bane for moose. *Oikos* 2001, 94, 442–454. [CrossRef]

38. Roelofs, J.G.M.; Schuurkes, J.A.A.R.; Smits, A.J.M. Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquat. Bot.* 1984, 18, 389–411. [CrossRef]

39. Svedang, M.U. Carbon dioxide as a factor regulating the growth dynamics of *Juncus bulbosus*. *Aquat. Bot.* 1992, 42, 231–240. [CrossRef]

40. Lucassen, E.C.H.E.T.; Roelofs, J.G.M.; Schneider, S.C.; Smolders, A.J.P. Long-term effects of liming in Norwegian softwater lakes: The rise and fall of bulbous rush (*Juncus bulbosus*) and decline of isoeidet vegetation. *Freshw. Biol.* 2016, 61, 769–782. [CrossRef]

41. Sand-Jensen, K. Environmental control of bicarbonate use among freshwater and marine macrophytes. In *Plant Life in Amphibious Habitats*; Crawford, R.M.M., Ed.; Blackwell Scientific Publications: Oxford, UK, 1987; pp. 99–112.

42. Hessen, D.O.; Ågren, G.I.; Anderson, T.R.; Elser, J.J.; De Ruiter, P.C. Carbon sequestration in ecosystems: The role of stoichiometry. *Ecology* 2004, 85, 1179–1192. [CrossRef]

43. Titus, J.E.; Andorfer, J.H. Effects of CO₂ enrichment on mineral accumulation and nitrogen relations in a submersed macrophyte. *Freshw. Biol.* 1996, 36, 661–671. [CrossRef]

44. Reitsema, R.E.; Preiner, S.; Meire, P.; Hein, T.; De Boeck, G.; Blust, R.; Schoelynck, J. Implications of climate change for submerged macrophytes: Effects of CO₂, flow velocity and nutrient concentration on *Berula erecta*. *Aquat. Ecol.* 2020, 54, 775–793. [CrossRef]

45. Elser, J.J.; Andersen, T.; Baron, J.S.; Bergstrom, A.K.; Jansson, M.; Kyle, M.; Nydick, K.R.; Steger, L.; Hessen, D.O. Shifts in lake N/P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. *Science* 2009, 326, 835–837. [CrossRef]
47. Ptacnik, R.; Solimini, A.G.; Andersen, T.; Tamminen, T.; Brettum, P.; Lepisto, L.; Willen, E.; Rekolainen, S. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. *Proc. Natl. Acad. Sci. USA* 2008, 105, 5134–5138. [CrossRef]

48. Smolders, A.; Lamers, L.; Lucassen, E.; Van der Velde, G.; Roelofs, J. Internal eutrophication: How it works and what to do about it—A review. *Chem. Ecol.* 2006, 22, 93–111. [CrossRef]

49. Rørslett, B. Aquatic weed problems in a hydroelectric river: The R. Otra, Norway. *Regul. Rivers Res. Manag.* 1988, 2, 25–37.

50. Moe, T.F.; Demars, B.O.L. Årsrapport Krypsivovervåking 2017; Report No L.NR. 7202-2017; NIVA: Oslo, Norway, 2017.

51. Rørslett, B. Tilgroing i Otra Nedstrøms Brokke. Problemanalyse og Forslag om Tiltak; Report no: O-86130; Norsk Institutt for Vannforskning: Oslo, Norway, 1987.

52. Murphy, K.J. Plant communities and plant diversity in softwater lakes of northern Europe. *Aquat. Bot.* 2002, 73, 287–324. [CrossRef]

53. Roelofs, J.G.M. Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands. I. Field observations. *Aquat. Bot.* 1983, 17, 139–155. [CrossRef]

54. Aulio, K. Elemental composition of *Juncus bulbosus* in an acidified freshwater reservoir. *Environ. Pollut.* 1987, 44, 1–11. [CrossRef]

55. Svedäng, M.U. The growth dynamics of *Juncus bulbosus* L.—A strategy to avoid competition? *Aquat. Bot.* 1990, 37, 123–138. [CrossRef]

56. Brandrud, T.E. Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquat. Bot.* 2002, 73, 395–404. [CrossRef]

57. Preston, C.D.; Pearman, D.A.; Dines, T.D. *New Atlas of the British & Irish Flora*; Oxford University Press: Oxford, UK, 2002.

58. Kaste, Ø.; Johansen, S.W.; Mjelde, M.; Andersen, T.; Hessen, D.; Holm, T.M.; Rangberg, A. Kan Næringsubalanse i Vann Føre til Problemvækst av Krypsiv? Resultater fra Forprosjekt i 2006; Report No 5341; Norsk Institutt for Vannforskning: Oslo, Norway, 2007.

59. R Core Team. *R: A Language and Environment for Statistical Computing* Vienna, Austria: R Foundation for Statistical Computing. 2018. Available online: http://www.R-project.org/ (accessed on 29 January 2021).

60. Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 2015, 67, 1–48. [CrossRef]

61. Barton, K. Package ‘MuMIn’. ‘R’ Package Version 1.15.62016. Available online: https://CRAN.R-project.org/package=MuMIn (accessed on 29 January 2021).

62. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. ImerTest: Tests in Linear Mixed Effects Models. R Package Version 2.0-292015. Available online: http://CRAN.R-project.org/package=ImeRTest (accessed on 29 January 2021).

63. Ter Braak, C.J.F.; Smilauer, P. *Canoco Reference Manual and User’s Guide: Software for Ordination, Version 5.0*; Microcomputer Power: Ithaca, NY, USA, 2012.