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Appropriate nutrient economy in *Phragmites australis* at different phases of estuarine succession

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Abstract: The common reed *Phragmites australis* (Cav.) Trin ex Steudel – the dominant macrophytic plant species in the Kokemäenjoki River delta, western Finland – showed distinct and appropriate trends in the nutrient economy according to the previously determined successional phases of the vegetation development. The height and weight of individual aboveground shoots (ramets) decreased in the order: Pioneer stage > Mature stage > Regressing stage. The concentrations of the major nutrients nitrogen and phosphorus were determined only partly by amounts of these nutrients in the plants' rhizospheres. The estuary is eutrophic, and the river water guarantees a continuous supply of nutrients, and thus the levels of nitrogen and phosphorus are high throughout the study area. The levels of nutrients correlated significantly with the contents of organic matter in the rhizospheres of the reed stands. On the basis of the relationships between the major nutrients' concentrations in the leaf blades, nitrogen appeared to be the growth-limiting nutrients in the pioneer stage of succession in these estuarine habitats. The N/P-ratios in the three stages were as follows: Pioneer stage: 10.75, Mature stage: 13.59, Regressing stage: 14.67. In general, the values below 15 are considered to be nitrogen-limited. The actual levels of N and P in the leaf tissues were, however, high throughout the study. As evaluated by the concept of critical concentration, i.e. the level of a nutrient, which guarantees maximal growth potential, the habitats at all the three successional phases showed adequate level of nutrient availability for the maximal production of *Phragmites*. At the pioneer stage, where the rhizosphere resources of nutrients were poorest, the common reed showed an appropriate morphological adaptation. In the pioneering stands, the common reed produces considerable amounts of adventitious roots (water roots) on the underwater sections of the culms. In the pioneer stage, the average weight of the water roots was 675 mg/ramet in the fertile (flowering) shoots and 267 mg/ramet in the sterile (non-flowering) shoots, i.e. 2.6–5% of the total weight of the aboveground shoots. In the mature stage, the amount of adventitious roots was minor, but in the regressing stage – where growth enhancement and substantial flowering is essential for the future of the species – the reeds produced more adventitious roots again.

Keywords: *Phragmites australis,* Common Reed, Successional Trends, Nutrient Economy, N/P-Ratio,
Adventitious Roots, Chlorophyll

1. Introduction

The common reed, *Phragmites australis* (Cav.) Trin ex Steudel is the most widely distributed flowering plant on the earth [1], and this fact alone is sufficient to prove the remarkable flexibility and adaptability of the species. In fact, the presence of *Phragmites* in any particular site does tell very little about the environmental conditions of the habitat: Due to the adaptability, the common reed can extend its presence even to the most unlikely extremes [2]. Both genetic and environmental factors are involved in the

development of morphological and functional adaptations – reflected in high plasticity – in *Phragmites australis* [3].

The mechanisms and efficiencies of nutrient utilization by plants are varied in environmental conditions, where the availability of resources is changing. Several studies on the utilization of nutrient resources in seashore vegetation during the course of primary succession showed that the principles often presented in the literature can be seriously simplified [4]. Variations in the resource availability in the

different phases of succession directly affect the growth and production abilities – and also determine the species composition of the dominant taxa in any specific habitat. The total amount and availability of nutrient resources are only a narrow determinant in controlling the success of competitive plants. Size and morphological characteristics – the architecture – of plants can be more relevant than nutrient utilization in determining the role and dominance of plant species in varying environmental conditions, typical for successional development of plant communities [4,5].

The dense and productive communities of littoral macrophytes provide valuable ecosystem services, through binding remarkable amounts of nutrients, and thus preventing harmful processes of eutrophication. The nutrient economy of any plant species and the stands of macrophytes are variable, and especially in heavily eutrophicated ecosystems the structure and function change often. The changes in the nutrient cycling and/or accumulation are in most cases unpredictable, and that is why detailed description of the local structure and state of within-stand development are necessary [6]. The role of productive stands of macrophytes is vital in determining both biological and physico-chemical features of aquatic ecosystems. By depositing autochthonous and nutrient-rich organic matter to the stands the reedswamp communities regulate and prevent water currents, and thus enhance the sedimentation processes of river-borne materials [7].

The ability of nutrient retention and translocation are important, especially in changing environments with varying supply of resources. Interspecific variations and differences between habitats are significant, and the views of the role of plant taxa are complex and sometimes opposite [8,9].

Plants can adapt to the invisible gradients in nutrient availability by utilizing appropriate variations in individual or species level. Such morphological and physiological adaptations include specific modifications in the growth and direction of rooting systems towards the best resources [10]. One of the alternatives for such adaptations is the development of adventitious roots to enhance the nutrient uptake in poor habitat conditions.

In spite of the facts that *Phragmites australis* is a cosmopolitan plant species, and the huge amount of research of the species, much is still to be examined about the real nature of the common reed. Comprehensive monographs and summarizations have been published, above all the works by Björk [11], Rodewald-Rudescu [12], and recently by Haslam [2]. Much is, however, still *terra incognita* – unknown and very little studied. The present study advances the knowledge about some appropriate strategies that the common reed exploits at the three previously determined successional phases in the Kokemäenjoki River delta, western Finland [13,14,15]. The main objectives to highlight specific strategies in the growth and production of the common reed were as follows:

- The utilization and accumulation of the major nutrients nitrogen and phosphorus, and the resulting N/P-ratios in the different habitat conditions.
- The development and role of adventitious roots (water roots) in the nutrient utilization as compared to the nutrient and organic matter concentrations of the rhizospheres.
- The concentrations of total chlorophyll (chl*a* + chl*b*) in the leaf blades in the three successional phases, and as related to the nutrient (especially nitrogen) availability in varying environments.

2. Materials and Methods

2.1. Study Area

The production and life history ecology of the common reed, *Phragmites australis* (Cav.) Trin ex Steudel were studied in the Kokemäenjoki River estuary, in western Finland (Northern Europe; 61°34'N, 21°40'E) in 1990's and again in 2013 (Figure 1). The estuary, discharging into the Baltic Sea, is thoroughly covered with rich and productive macrophytic vegetation, and the stands of tall helophytic plants form distinctly separable successional zones (Figure 2). In *Phragmites australis*, three successional phases – Pioneer stage, Mature stage and Regressing stage – were separated and described. The variable characteristics of the estuary, the Pihlavanlahti Bay, were described in detail previously [14,15].

Figure 1. *Location of the study area.*

2.2. Collection and Analyses of the Plant Materials

The plant samples of *Phragmites australis* were collected by the time of the maximum biomass of reeds (August – early September). The sampling was made randomly in the middle of uniform, monocultural stands at three successional phases [14,15]. The biometric measurements, as well as the frequency of the flowering ramets were determined for 200–250 randomly sampled individuals at each of the successional stages. The water depth of the sites was determined by 10 measurements in each site. The sampling and measurement policies followed the international standards used in hydrobiological studies [16].

For chemical analyses, wet-digestion methods were used [17]. Dried and pulverized samples were digested with

sulphuric acid-hydrogen peroxide reagent. The concentrations of nitrogen were determined by semi-micro Kjeldahl distillation and titration with 0.01 N sulphuric acid. Mixed acid reagent was used in the digestion for phosphorus analyses. The concentrations of phosphorus were determined colorimetrically by using the ammonium molybdate method [17].

The contents of organic matter of the sediment samples were determined after dry ashing (at 475 ^{o}C, 4 h). All the results are expressed on a dry weight basis. For plant and sediment dry matter determinations, the samples were oven-dried at 105 ^{o}C for 48 h. The redox potential of the sediments was determined directly in the field by using a glass electrode [16]. The concentrations of the total chlorophyll (chl *a* + chl *b*) in fresh *Phragmites* leaf blades were determined after a DMSO extraction method presented by Hiscox and Israelstam [18]. The chlorophyll concentrations were calculated according to Arnon [19].

2.3. Statistical Analyses and Terminology

The statistical analyses used follow Sokal and Rohlf [20]. The parametric (mean ± standard deviation of the mean, one-way analysis of variance; ANOVA), and non-parametric (Kruskal–Wallis one-way analysis of variance) statistical analyses of the numerical data were performed by using the *Analyse-it for Microsoft Exel* (version 2.12) program package [21].

The terminology of biological concepts and principles follows the latest edition of the *Oxford Dictionary of Plant Sciences* [22].

3. Results and Discussion

3.1. Size of the Aboveground Shoots

The growth and production conditions of aquatic and littoral macrophytes can vary significantly within a habitat, thus making direct comparisons and any generalizations difficult, often impossible. Rapid changes and marked microhabitat differences are typical in coastal areas, especially in river deltas and estuaries. Continuous change is even more advanced in the estuaries on the coasts with land upheaval. The Kokemäenjoki River estuary in western Finland is one of the most striking examples of the mosaic and continuous change because the succession of macrophytic vegetation is more rapid than in any other habitat in Northern Europe [14,15].

In the previous studies, three successional phases were separated and described for the dominant tall helophytic plant of the area, the common reed *Phragmites australis*. The characteristics of *Phragmites* differ significantly between the Pioneer, Mature, and Regressing phases of succession. Eight biometric parameters were determined and compared in the previous studies [14,15]. Results of the height and weight of *Phragmites australis* aboveground shoots (ramets) in the Kokemäenjoki River delta are summarized in Table 1.

Table 1. Summary of the height and weight comparisons of the flowering (fertile) and non-flowering (sterile) shoots of Phragmites australis in the three successional stages in the Kokemäenjoki River delta, western Finland (data compiled from Aulio 2014, a,b).

	Pioneer	Mature	Regressing
Fertile shoots			
● Height, cm	266.93 ± 20.06[a]	262.94 ±27.17[a]	188.34 ± 10.42[b]
● Weight, g (dry wt)	15.49 ± 1.22[a]	10.35 ± 1.11[b]	5.71 ± 0.60[c]
Sterile shoots			
● Height cm	177.21 ± 24.14[a]	174.03 ±16.83[a]	142.43 ± 16.67[b]
● Weight, g (dry wt)	9.62 ± 2.01[a]	6.51 ± 1.86[b]	4.32 ± 1.67[c]

Mean ± standard deviation (S.D.). *N* = 200–250 ramets in height and 50–75 ramets in weight determinations in each successional stage. Statistical significance of the differences: In ANOVA, the different superscript letters in the horizontal rows indicate highly significant difference (*P* < 0.01).

Figure 2. The dense monocultures of Phragmites australis cover thousands of hectares at the mature stage of succession in the Kokemäenjoki River delta, western Finland.

3.2. Nutrient Status of the Habitats

Fertility of the habitat is one of the key factors in determining the production potential of plants. Different plant species utilize the environmental resources differently, and the relations between the nutrient levels often determine the dominance in the ecosystem. In wetland ecosystems, the role of the major nutrients nitrogen and phosphorus can vary significantly. In terrestrial ecosystems, nitrogen is most commonly the growth-limiting nutrient, and the same applies to many wetlands, especially in the driest habitat types. In moist and aquatic conditions, the availability of phosphorus is mostly the critical factor in limiting primary production, especially in phytoplankton but also in macrophytes.

Nutrient utilization by plants is very variable, however, and many wetland plants suffer from deficiency of nitrogen [7]. The present results obtained from the Kokemäenjoki River delta show that the concentrations of the major

nutrients are high throughout the study area – in all the three successional phases of *Phragmites australis* – as compared to the typical values for emergent wetland plants growing in temperate climatic conditions, even those heavily fertilized with nitrogen, phosphorus and potassium [7,23,24].

The present study area, the Kokemäenjoki River estuary, western Finland, is classified as eutrophic, or even polluted by anthropogenic emissions, and thus the availability of nutrients should not be limiting factor the success of macrophytes. The environmental conditions prevailing at the estuary are, however, very variable and changing, and differences in the habitat characteristics between the successional phases of the reedswamps are significant [14], [15]. The nutrient concentrations and availability differ markedly in waters and especially in the sediments at the macrophytes' rhizospheres, thus influencing crucially the development and maintenance of macrophytic vegetation.

The contents of organic matter, and the major nutrients nitrogen and phosphorus at the three successional phases of *Phragmites australis* in the Kokemäenjoki River delta are presented in Table 2. The levels of organic matter and nutrients were high throughout the study sites. The nutrients are bound with the organic matter, thus determining the fertility of each habitat. In the Pihlavanlahti Bay, the levels of N and P in the sediments correlated highly significantly with the contents of organic matter (principally the autochthonous debris of the sites' own plant material). In the whole study, the Pearson correlation between the levels of nitrogen and organic matter was r = 0.934 ($P < 0.001$), and accordingly between phosphorus and organic matter, r = 0.852 ($P < 0.001$), $df = 15$. The characteristics of the rhizospheres differ, however, significantly between the successional phases, thus leading to marked variations in the biometric measures and production abilities of the common reed.

Table 2. The concentrations of organic matter, nitrogen, and phosphorus in the rhizospheres of Phragmites australis at the three successional stages in the Kokemäenjoki River delta, western Finland. All the values are expressed on the dry weight basis (Aulio (2014a).

	Pioneer	**Mature**	**Regressing**
Organic matter, %	2.84 ± 1.17[a]	10.18 ± 1.09[b]	21.41 ± 1.21[c]
Nitrogen, mg/g	1.79 ± 0.40[a]	5.21 ± 0.41[b]	6.57 ± 0.35[c]
Phosphorus, mg/g	0.30 ± 0.05[a]	0.92 ± 0.09[b]	0.99 ± 0.54[b]

Mean ± standard error of the mean (S.E.). $N = 15–20$ samples in each successional stage. Statistical significance of the differences: In ANOVA, the different superscript letters in the horizontal rows indicate highly significant difference ($P < 0.01$).

The strong correlations between the contents of organic matter and the major nutrients are due to the sediment properties. The rhizospheres are very rich in nutrients in the study area, and in such conditions the decomposition of organic matter is more efficient than in oligotrophic conditions. In eutrophic sediments, i.e. conditions like those in the Kokemäenjoki River delta, the levels of nitrogen remain high or even increase due to the amount of

bacteria present in the rhizosphere [25]. In contrast, in oligotrophic habitats the sediment layer is often impoverished due to the leaching of elements.

The relationship between the major nutrients is important in determining the maintenance and success of plants in their natural environments. The ratio between the contents or availability of nitrogen to phosphorus is often used as an indicator in determining the ecological control of plants. In the present study, the N/P -ratios prevailing in the rhizospheres of *Phragmites australis* differed markedly between the three successional phases.

The average N/P-ratio was 5.97 in the pioneer stage, 5.66 in the mature stage, and highest ratio was determined in the regressing stage, 6.64 [14]. These values of the nutrient ratios are typical for lakes in the North Europe, but the trend in the differences between the successional phases was special at the Pihlavanlahti Bay. In the reedswamps in Swedish lakes, the N/P-ratios were highest in the outermost stands towards the open water, and accordingly, in the shallow-water stands near the shoreline, this ratio was the lowest of the comparison [26]. Low N/P-ratio often shows that the rhizosphere in the littoral habitat is – or at least has been – anaerobic at least during the hottest weeks of summer and early autumn (in August-September in the Northern Europe). In anaerobic conditions, the microbial processes of denitrification reduce the amount of plant-available nitrogen nutrients.

The role of denitrification processes seems to be minor in the present study area. In the pioneer stage of *Phragmites* succession the rhizosphere is never anoxic, due to continuous water exchange, coarse sediment composition, and low contents of organic matter. In the mature, and especially in the regressing stages of succession, the contents of organic matter were up to three times the values prevailing in the pioneer stands. Here the microbiological decomposition of organic matter consumes the oxygen reserves of the rhizosphere, and at least during the hottest weeks of the growing season the sediment layer in the rhizosphere suffers from low oxygen status – even from totally anaerobic conditions. In the regressing successional phase, the field measurements (*in situ*) of redox potential proved that reducing conditions prevailed. E_h values in the redox measurements were typically between -50 – -200 mV during the peak summer season.

The dense and very productive stands of *Phragmites australis* in the Kokemäenjoki River delta can change the environmental conditions through their own activities, especially in the sheltered, shallow water habitats of the mature and regressing successional phases. The local variations between the three successional phases are significant. On the basis of the contents of organic matter and the major nutrients in the rhizosphere, the three successional phases differed statistically highly significantly from each other. In the nonparametric Kruskall-Wallis one-way analysis of variance, $\chi^2 = 24.47$, $P < 0.0001$, $df = 2$. Similarly, highly significant variations were recorded in the comparisons between the nutrient

contents of the rhizospheres at the three successional stages. In the comparison between the nitrogen levels, $\chi^2 = 21.14$, $P < 0.0001$, and accordingly, between the contents of phosphorus, $\chi^2 = 18.79$ ($P < 0.0001$, $df = 2$.

3.3. Major Nutrients in Reed Tissues

The utilization of nutrients varies widely between plant taxa – even in closely related species. In the comprehensive review of literature, Verhoeven *et al.* [27] summarized the results of 45 studies on nutrient concentrations in herbaceous wetland plants in temperate climatic conditions. The average relation between the major nutrients – N/P-ratio – in the aboveground biomass at the end of the growing season was 15:1. The values of N/P-ratio below 15 would thus indicate nitrogen limitation, and accordingly, ratios greater than 16 would indicate phosphorus limitation in macrophytic plant production [27].

In the present study at the Pihlavanlahti Bay, the average N/P-ratios in the leaf blades of *Phragmites australis* were as follows: Pioneer stage: 10.75, Mature stage: 13.59, Regressing stage: 14.67. On the basis of these nutrient relationships, the stands of *Phragmites* in the pioneer phase of succession seem to be clearly nitrogen limited, whereas the other two phases show nutrient relationships close to optimal ratios.

The role of nutrients is, however, only seldom the limiting factor in the growth and production ecology of macrophytic plants in eutrophic aquatic environments. The nutrient status is the decisive factor in determining the success of *Phragmites australis* only in nutrient-poor waters and wetlands [2,28]. In oligotrophic lakes, however, the nitrogen status of the plants' rhizosphere and especially of the ambient water can be the crucial growth-limiting factor for the common reed [29].

Table 3. *The concentrations of the major nutrients nitrogen and phosphorus in the leaf blades and stem (culm) tissues of Phragmites australis at the three successional stages in the Kokemäenjoki River delta, western Finland.*

Successional stage	Nitrogen, %	Phosphorus, %
Pioneer		
● leaf blades	2.58 ± 0.16^a	0.24 ± 0.10^a
● culms	0.14 ± 0.07	0.08 ± 0.06
Mature		
● leaf blades	2.31 ± 0.20^b	0.17 ± 0.05^b
● culms	0.11 ± 0.06	0.04 ± 0.02
Regressing		
● leaf blades	2.20 ± 0.48^b	0.15 ± 0.04^b
● culms	0.12 ± 0.08	0.03 ± 0.01

Mean ± standard error of the mean (S.E.). $N = 15–20$ samples in each successional stage. Statistical significance of the differences: In ANOVA, the different superscript letters in the vertical columns indicate highly significant difference ($P < 0.05$).

Aquatic primary producers take up and accumulate nutrients in their tissues to ensure maximal growth and physiological maintenance processes. In macrophytes, the average ratio between the major nutrients nitrogen and phosphorus tied in plant tissues is 1 : 10–15 [30]. The levels of nutrients in the physiologically active leaf tissues in *Phragmites australis*, collected from the Kokemäenjoki River delta show that nitrogen is more readily available for primary producers than phosphorus nutrients. Nutrient ratios are, however, very variable at the three successional phases.

In general, the levels of the major nutrients N and P in the leaf blades and stems (culms) of *Phragmites australis* at the Pihlavanlahti Bay were high (Table 3).

The contents of nitrogen and phosphorus in the *Phragmites* leaves were higher than the critical concentration value, determined for aquatic plants by Gerloff and Krombholz [30]. The concept of critical concentration refers to the level of a nutrient, which ensures the maximal growth and physiological functions. The critical concentration value in angiosperm aquatic plants for nitrogen is 1.3 %, and for phosphorus the level is 0.13% (on the dry weight basis). When these values are applied, the major nutrients are not growth-limiting in the reedswamps in the Kokemäenjoki River delta. Local variations and exemptions do exist, however.

Major nutrients are so readily available in the Pihlavanlahti Bay that the contents of nitrogen in the leaf blades of *Phragmites* were more than two per cent (dry weight) in all three successional phases, thus exceeding the critical concentration threshold nearly twofold in all the samples studied. The levels of phosphorus (0.15–0.24 %, on average) were also high, as compared to the average values reported for emergent wetland plants in other studies. In the pioneer stage, the enrichment of phosphorus was marked, even though the contents of P in the rhizospheres were rather low (cf. Table 2).

The levels of N and P were very low in the culms throughout the study, but in these values the concept of critical concentration cannot be applied. The stems are mostly metabolically passive structural tissues, where the nutrient relations are determined by other basis than in the photosynthetically active leaf tissues.

The highest contents of nitrogen and phosphorus were determined in the pioneer stage of succession (Table 3). As compared to the results published elsewhere, this trend may seem surprising because at the pioneer phase the contents of major nutrients in the rhizospheres were significantly lower than at the other two phases. The minerotrophic sediments at the pioneer stage cannot accumulate and hold nutrients efficiently. In the Kokemäenjoki River delta, the common reed reacts to the lower availability of sediment-borne nutrients by an appropriate morphological adaptation. To compensate the nutrient scarcity in the rhizosphere, *Phragmites* had a marked growth of adventitious, water roots on the underwater stems (Figure 3).

3.4. Adventitious Roots in Varying Habitats

In nutrient-poor habitats, adventitious roots are sometimes grown to enhance nutrient acquisition capacity. In *Phragmites australis*, such structures are developed on the underwater sections of the stems, above the surface of sediment layer. The role of the adventitious roots (water

roots) is enhanced in situations, where the reeds occupy sandy bottoms with very low nutrient concentrations [12,31]. Such an appropriate investment of resources is seen in communities of *Phragmites australis* in the Kokemäenjoki River delta.

Figure 3. *Adventitious roots (water roots) are important in enhancing nutrient uptake of Phragmites australis growing on the poorest sediments.*

As compared to the other successional stages, the amount of water roots was markedly enhanced at the pioneer phase (Table 4).

Water roots or adventitious roots are structures developed into positions, where roots are not normally found in plants. In aquatic and littoral helophytic plants, water roots develop into the stems above the sediment/water interface, permanently submerged in water. In *Phragmites australis*, water roots appear only in habitats, where the plant or clone is flooded during the growing season [2].

Table 4. *The occurrence of adventitious roots (=water roots) in flowering and non-flowering Phragmites australis shoots at the three successional stages in the Kokemäenjoki River delta, western Finland. The total amount and proportion of water roots of the aboveground shoot.*

Successional stage	Number of samples/ group	Weight of adventitious roots, g dry wt/ramet	Share of adv. roots of the ramet's mass, %	Statistical difference within the groups
Pioneer				
● Fertile	56	676.5 ± 108.2^a	2.59 ± 2.61	$F = 5.29$ ($P=0.024$)
● Sterile	23	267.6 ± 85.2^b	4.96 ± 4.85	
Mature				
● Fertile	44	29.2 ± 7.4^a	0.10 ± 0.08	$F = 6.58$ ($P=0.012$)
● Sterile	28	5.3 ± 1.5^b	0.08 ± 0.13	
Regressing				
● Fertile	46	15.9 ± 2.8^a	0.16 ± 0.18	$F= 1.12$ ($P=0.291$)
● Sterile	21	12.5 ± 3.9^a	0.24 ± 0.33	

Mean ± standard error of the mean (S.E.). Statistical significance of the differences: In ANOVA, the different superscript letters in the vertical columns indicate highly significant difference ($P < 0.01$).

The primary purpose of producing adventitious roots in plants is to overcome oxygen deficiency [32]. In *Phragmites australis* this task is, however, of secondary importance, because the reed has extensive aerenchyma (internal, porous structures and channels for air transport from the atmosphere to different organs, also to the belowground organs) in the stems, rhizomes and roots [2,12,33].

The occurrence of adventitious roots in tall littoral macrophytes is a poorly studied phenomenon. In submerged taxa and in terrestrial plants different kinds of adaptations in rooting structures are more studied. Comparisons show the variations are wide and species-specific, and dependent of the plants' habitat characteristics [34].

In the present study, the occurrence and weight of the adventitious roots in the submerged sections of stems of *P. australis* correlated with the total mass of the ramets (shoots). The frequency and mass of the adventitious roots differed markedly between the successional stages, however (Table 4).

The mass of the adventitious roots was overwhelmingly highest in the ramets at the pioneer stage, 572.2 mg/ramet, on the average. But marked variations were seen even within the successional classes. Thus, in the pioneer stage, mass of adventitious roots was more than double in fertile (676 mg/ramet) as compared to the sterile ramets (268 mg/ramet). The difference between the flowering and non-flowering ramets was statistically significant ($P < 0.01$).

In the established mature stands, the mass of adventitious roots in the stems of the common reed was 10.8 mg/ramet, on the average. In flowering shoots, the mass of adventitious roots was 29.2 mg/ramet, but in the sterile shoots the mean mass was only 5.3 mg/ramet. Towards the final stage of the successional development, the stands invested more in the adventitious roots, again. At this phase the mean mass of the water roots was 14.88 mg/ramet. In the flowering shoots the mean mass of water roots was 15.86 mg/ramet, and in the sterile shoots the mass was nearly the same, 12.46 mg/ramet (Table 4).

Die-back of monocultures of *Phragmites australis* – widely observed in Europe – is reflected both in the morphological and physiological features of the shoots. In the Kokemäenjoki River delta, the height and density of the common reed clearly reflect successional stages of the macrophytic vegetation [14,15]. The present results of the occurrence and masses of adventitious roots on the aboveground, underwater stems of *P. australis* are consistent with the observations from southern Europe. Reale *et al*. [35] showed the interrelationship between the water roots and the die-back of the reed communities. The decline in the physiological status and photosynthetic capacity as related to the die-back was seen in a weakened storage of starch in the adventitious roots [35].

In the wide selection of Swedish lakes, the distribution of water roots in *Phragmites* showed varying patterns. In

general, fine roots were most common in the uppermost layer of sediments, but water roots were also seen in the water column. But contrary to the present study, in the Swedish lakes the basal nodes of the common reed were often richly covered with bushy fine roots especially in the deep water habitats and in lakes with a relatively low electrolyte concentrations [11]. In the Kokemäenjoki River delta, the water roots at the bases of the reeds' culms were most often found in the stands along the river and estuary channels, where the supply of waterborne nutrients is continuous and abundant.

The stems and roots – also adventitious roots – of *Phragmites australis* have exceptionally high volume of aerenchyma, and thus the oxygenation of inundated and belowground stems and rhizosphere is well-developed [2]. In the interspecific comparison, the porosity (i.e. the volume and proportion of intercellular gas spaces and aerenchyma) in the water roots of *Phragmites australis* was

overwhelmingly the highest among the eight species of wetland plant species and eight non-wetland plants compared [32,36].

3.5. Chlorophyll Content in Leaf Tissues

The role of nutrient availability in the growth regulation and well-being of macrophytes is clearly shown in the comparison between the leaf nitrogen and phosphorus levels and the contents chlorophyll in the leaf blades. The general assumption is that the contents of chlorophyll, as well as the production potential of *Phragmites* are maximal in habitats, where the nutrient resources are rich [24,37]. In the present study, the levels of chlorophyll reflected the nutrient contents of the leaves, but the relationship between the chlorophyll and the nutrient status of the plants' rhizospheres showed unexpected trends (Table 5).

Table 5. *The concentrations of chlorophyll (chla + chlb) in the leaf blades of Phragmites australis at the three successional stages in the Kokemäenjoki River delta, with the contents of major nutrients and organic matter in the rhizospheres of the reeds.*

Successional stage	Chlorophyll *a + b*, mg/g fresh weight	Nitrogen, mg/g dry weight	Phosphorus, mg/g dry weight	Organic matter, mg/g dry weight
Pioneer	1.87 ± 0.22	2.25 ± 1.28	0.38 ± 0.19	1.75 ± 1.18
Mature	1.45 ± 0.09	4.66 ± 0.62	1.09 ± 0.09	9.03 ± 1.89
Regressing	1.26 ± 0.13	5.99 ± 0.69	0.96 ± 0.12	18.48 ± 2.74

Mean ± standard error of the mean (1 S.E.). $N = 12$ in each successional stage.

The chlorophyll contents of the leaf blades reflect directly the production potential of *Phragmites.* Hence, the highest contents of chl*a*+chl*b* were determined in the pioneer stage of succession, i.e. in the stands with the tallest and heaviest plants, and in individuals with the highest nutrient contents in the leaf blades. The levels of chlorophyll showed a distinct pattern according to the successional phases of the common reed. The biometric dimensions (size of the ramet, with eight parameters determined previously [14]), and the leaf chlorophyll contents decreased in the order: Pioneer > Mature > Regressing stages. The trend was statistically significant ($P < 0.01$).

The trend in the chlorophyll levels could be considered somewhat surprising, as the highest contents were determined in the plants growing on the poorest sediments. Usually, the content of plant leaf chlorophyll reflects the nutrient status, especially the amount and availability of nitrogen. Of the total amount of organic nitrogen, up to 75 % is concentrated in chloroplasts and metabolically active enzymes [38]. The anomalous results in the present study can be expressed to the pattern of adventitious roots, absorbing nutrients directly from the aqueous environment. The river water surrounding the reed plants at the pioneer stage is eutrophic, and concentrations of various nitrogen sources are high throughout the growing season. On the other hand, the low concentrations of chlorophyll at the regressing stage directly reflect the reduced growth potential in these die-off stands.

The levels of leaf chlorophyll were negatively correlated with the nitrogen, phosphorus and organic matter contents of

the rhizospheres (Table 5). In the case of nitrogen, the negative relationship was the most striking (r = -0.804, $P < 0.001$), and the other two correlations were negative, too ($P < 0.05$). The negative correlations between the chlorophyll contents and the nutrients and organic matter concentrations prevailing in the rhizospheres are logical due to the chemical forms of nutrients (especially N) in the aquatic environment. The majority of the total nitrogen (as analyzed in the present study) in the soil and sediments is tightly bound to organic matter, and becomes available to primary producers only after microbiological transformations.

4. Summary and Conclusions

The biometric characteristics in the monotypic communities of *Phragmites australis* in the Kokemäenjoki River delta, western Finland showed distinctive patterns according to the three previously determined successional phases. In the comparisons of the nutrient economy of the common reed, following trends were observed:

(1) The height and weight of individual shoots (ramets) of the common reed decreased in the order: Pioneer stage > Mature stage > Regressing stage. Similar – and biologically appropriate – trends were seen in the nutrient uptake and accumulation capabilities of *Phragmites* leaves.

(2) The uptake/accumulation of the major nutrients nitrogen and phosphorus were efficient at all the successional phases studied. On the basis of the relationships between the major nutrients, nitrogen

appeared to be the most obvious growth-limiting nutrient, but the results showed that such a limitation only sporadically occurs in the eutrophic estuary.

(3) The total amount of nutrients in the plants' rhizospheres was only poorly reflected in the nutrient concentrations of the leaf blades. The concentrations of nitrogen and phosphorus correlated highly significantly with the contents of organic matter in the sediment layers. Majority of nutrients – especially nitrogen – are tightly bound with organic matter, and so the total level of resources only partly mirror the actual availability of nutrients to the primary producers.

(4) The concentrations of N and P in the rhizospheres were high throughout the study, but variations were seen according to the successional phases. The lowest N/P-ratio was seen at the pioneer phase, and highest one at the regressing, die-off phase.

(5) On the basis of the relationship between the major nutrients accumulated in the leaf blades – the N/P-ratio – nitrogen limitation is apparent in the pioneer phase of succession. This is surprising because the growth and production of *Phragmites* peaks in these stands. In the mature and regressing phases, the N/P-ratios were close to the optimal values, reflecting maximal production capacity as far as nutrients are concerned.

(6) The actual levels of nitrogen and phosphorus in the leaf blades exceeded the critical concentration values, thus showing that the nutrient availability in the study area allows maximal growth for *Phragmites australis* at all the successional phases compared in the study area.

(7) The high levels of nutrients in the ramets growing on the poorest sediments could be explained with a morphological adaptation. *Phragmites* shows an appropriate strategy by growing extensive adventitious roots (water roots) when growing on sandy sediments at the pioneer phase.

(8) The role of nutrient availability – especially that of nitrogen – was clearly proved in the comparison of the chlorophyll concentrations in the leaf blades of the common reed. The levels of chlorophyll reflect straightly the growth and production capacity of *Phragmites australis*, and the levels appropriately followed the trend of the biometric components at the three successional stages.

References

[1] Stott P. 1981. Historical Plant Geography. pp. 151. George Allen & Unwin, London.

[2] Haslam SM. 2010. A Book of Reed. pp. 261. Forrest Text, Swn y Nant.

[3] Eller F and Brix H. 2012. Different genotypes of *Phragmites australis* show distinct phenotypic plasticity in response to nutrient availability and temperature. Aquatic Botany 103: 89–97.

[4] van der Veen A. 2000. Competition in coastal sand dune succession. Cause or mechanism? pp. 126. Doctoral dissertation. University of Groningen (Rijksuniversiteit Groningen).

[5] Elberse WT and Berendse F. 1993. A comparative study of the growth and morphology of eight grass species from habitats with different nutrient availabilities. Functional Ecology 7(1): 223–229.

[6] Khan FA and Ansari AA. 2005. Eutrophication: An ecological vision. The Botanical Review 71(4): 449–482.

[7] Keddy PA. 2010. Wetland Ecology. Principles and Conservation, Second Edition. pp. 497. Cambridge University Press. Cambridge.

[8] Berendse F and Aerts R. 1987. Nitrogen-use-efficiency: a biologically meaningful definition? Functional Ecology 1(3): 293–296.

[9] Eckstein RL. 1999. Nutrient use strategies of plants of various life-forms in a subarctic environment. Nutrient conservation as an adaptation to infertile habitats. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 490: 1–32.

[10] Grime JP. 2001. Plant Strategies, Vegetation Processes, and Ecosystem Properties, Second Edition. pp. 417. John Wiley & Sons, Chichester.

[11] Björk S. 1967. Ecologic investigations of *Phragmites communis*. Studies in theoretic and applied limnology. Folia Limnologica Scandinavica 14: 1–248.

[12] Rodewald-Rudescu L. 1974. Das Schilfrohr *Phragmites communis* Trinius. Die Binnengewässer 27: 1–302.

[13] Aulio K. 2010. The Kokemäenjoki River: A success story in water conservation. Baltic Cities Environmental Bulletin 2/2010: 7.

[14] Aulio K. 2014a. Strategies in growth of the common reed (*Phragmites australis*) as related to successional stages in a rapidly varying estuary. Research Journal of Biology 2: 11–17.

[15] Aulio K. 2014b. Allocation to sexual reproduction by the common reed (*Phragmites australis*) is highly variable in different phases of estuarine succession. Research Journal of Biology 2: 53–59.

[16] Vollenweider RA (Ed.). 1969. A manual on methods for measuring primary production in aquatic environments. pp. 213. IBP Handbook 12. London.

[17] Allen SE (Ed.). 1974. Chemical Analysis of Ecological Materials. pp. 565. Blackwell, Oxford.

[18] Hiscox JD and Israelstam GF. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany 57(12): 1332–1334.

[19] Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiology 14(1): 1–15.

[20] Sokal RR and Rohlf FJ. 2012. Biometry. Fourth Edition. pp. 937. W.H.Freeman and Company, New York.

[21] Analyse-it Software Ltd. 2008. Analyse-it for Microsoft Exel (version 2.12). http://www.analyse-it.com

[22] Allaby M. (Ed.). 2012. Oxford Dictionary of Plant Sciences. Third Edition. pp. 565. Oxford University Press, Oxford.

[23] McJannet CL, Keddy PA and Pick FR. 1995. Nitrogen and phosphorus tissue concentrations in 41 wetland plants: a comparison across habitats and functional groups. Functional Ecology 9(2): 231–238.

[24] Rickey MA and Anderson RC. 2004. Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. Journal of Applied Ecology 41(5): 888–896.

[25] Andersen FØ. 1978. Effects of nutrient level on the decomposition of *Phragmites communis* TRIN. Archiv für Hydrobiologie 84: 42–54.

[26] Andersson B. 2001. Macrophyte development and habitat characteristics in Sweden's large lakes. Ambio 30(8): 503–513.

[27] Verhoeven JTA, Koerselman W and Meuleman AFM. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. Trends in Ecology and Evolution 11(12): 494–497.

[28] Haslam SM. 1971. Community regulation in *Phragmites communis* Trin. I. Monodominant stands. Journal of Ecology 59(1): 65–73.

[29] Szczepański A. 1978. Ecology of macrophytes in wetlands. Polish Ecological Studies 4: 45–94.

[30] Gerloff GC and Krombholz PH. 1966. Tissue analysis as a measure of nutrient availability for the growth of angiosperm aquatic plants. Limnology and Oceanography 11(4): 529–537.

[31] Haslam S. 1973. Some aspects of the life history and outecology of *Phragmites communis* Trin. A review. Polskie Archiwum Hydrobiologii 20: 79–100.

[32] [32] Gregory PJ. 2008. Plant Roots: Growth, Activity and Interactions with the Soil. pp. 328. Blackwell Publishing, Oxford.

[33] [33] Armstrong J and Armstrong W. 1988. *Phragmites australis* – A preliminary study of soil-oxidizing sites and internal gas transport pathways. New Phytologist, 108(4): 373–382.

[34] [34] Stevens KJ and Peterson RL. 2007. Relationships among three pathways for resource acquisition and their contribution to plant performance in the emergent aquatic plant *Lythrum salicaria* (L.). Plant Biology 9(6): 758–765.

[35] Reale L, Gigante D, Landucci F, Ferranti F and Venanzoni R. 2012. Morphological and histo-anatomical traits reflect die-back in *Phragmites australis* (Cav.) Steud. Aquatic Botany 103: 122–128.

[36] Colmer TD. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell and Environment 26(1): 17–36.

[37] Bornkamm R, Raghi-Atri F and Koch M. 1980. Einfluss der Gewässereutrophierung auf *Phragmites australis* (Cav.)Trin. ex Steudel. Garten und Landschaft 1/80: 15–19.

[38] Marschner H. 1995. Mineral Nutrition of Higher Plants. Second Edition. pp. 889. Academic Press. London.