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Site-Effects Dominate the Plant Availability of Nutrients under Salix Species during the First Cutting Cycle

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Abstract: Fast-growing willows (*Salix* spp.) provide alternative sources of renewable energy generation, but need an adequate nutrient availability in the soil for high biomass production. In general, species mixtures can be more nutrient-efficient than pure cultures, but this is scarcely known for *Salix* spp. Therefore, this study evaluates the nutrient availability and P mobilization under two willow species, *Salix dasyclados* var. 'Loden' and *S. schwerinii* × *viminalis* var. 'Tora', grown as pure and mixed cultures at non-fertilized former arable sites in Germany (Stagnic Cambisol) and Sweden (Vertic Cambisol). The plant availability of potassium (K), magnesium (Mg) and phosphorus (P) and soil phosphatase activities in the topsoil were measured in spring of the year of planting (initial) and under 4 years-old stocks (one year after the first 3-year cutting cycle). The initial plant availability of the nutrients significantly differed between the sites and the two sampling dates at both sites. The plant availability of K and Mg was optimal to high at both sites and sampling dates, but rather low for P (after 4 years $\leq$5 mg P 100 g^{-1} soil). The plant-available P and K content in soil significantly decreased within the 4 years of willow growth at both sites. The acid and alkaline phosphatase activity in the soil of the German site (Rostock) was significantly lower after 4 years of willow growth, but differed not significantly between the two sampling dates at the Swedish site (Uppsala). Higher activity of acid phosphatase compared to alkaline phosphatase was recorded in the soils at both test sites based on the site-specific soil pH (<7). The slight decrease of plant availability of P after 4 years of *Salix* growth in pure culture differed not significantly between the different species. Mixed growth did not decrease the plant availability of P within this period, although no significant difference in the biomass production of pure and mixed growth was observed. This was valid at both sites, and therefore, seems independent of the site-specific differences in soil and climate conditions. The general validity of the assumptions should be tested also for other species mixtures and soil conditions in the future before site-adapted growth designs can be recommended in biomass production of *Salix*.

Keywords: short rotation coppice; phosphatase activity; nutrient content; growth stages; biomass; willow; *Salix*

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1. Introduction

Short rotation coppices (SRCs) with poplar (*Populus*) and willows (*Salix*) species can be established on many types of land, including marginal lands that are unsuitable for agriculture [1,2]. SRCs offer a promising contribution to fuel wood supply, providing an alternative to fossil fuels and other nonrenewable resources [3]. The harvest from SRCs are usually used as feedstock in combined heat and power plants for energy generation [4,5]. Moreover, SRC plantations have ecological benefits compared to annual crops. They improve the soil water retention, enhance biodiversity in comparison to agricultural monocultures, improve water quality, protect ground water, prevent soil erosion and it is a low-input agricultural practice, thus implying low greenhouse gas emissions due to limited applications of chemicals [6–10] SRCs have been investigated intensively for the last 20 years (reviewed by Rödl [11]). SRC are managed using agricultural techniques, including high-density plantings and a regular cutting every 2 to 6 years without replanting [12]. The choice of tree species for SRC is generally confined to fast-growing tree species, such as those from the genera *Populus* and *Salix* [13]. In Europe, poplar and willow (*Populus* and *Salix*) belong to the natural vegetation of the softwood floodplain forest [14,15]. Poplar is typical for Western and Central European floodplains, where willow is more tolerant to low temperatures and can grow further north and in continental climates in the east of Europe [16].

Willows are deciduous trees or shrubs and comprise 330–500 species around the world [17]. They adapt in cool climates and high altitudes or on wet soils [18]. Willows require sufficient moisture supply during site establishment, while in later plantation stages, they can adapt to dry environments with heat and drought stress [17]. Studies have proven that willows have high tolerance to marginal or contaminated soils [6,8,19]. Willow SRCs are gaining increasing interest, because of their efficient and sustainable land use in combination with a growing demand for biofuel resources [20]. In Sweden, willow coppices are often used for phytoremediation where wastewaters or sewage sludge is applied to plantations in order to reduce pollutants or excess nutrients in the water [21].

Previous studies have reported that the productivity in SRCs is determined mainly by the soil fertility [22], soil pH (usually 5–7.5, but willow and poplar are tolerant to pH outside this range) [23], climatic conditions, nutrient and water availability [24], plant species and plantation density [25]. The need for fertilizers in SRCs is small compared to conventional agricultural crops [26]. SRC yields can be maximized by establishing them at fertile soils or by applying organic fertilizers, such as slurry, digested sewage sludge, manure from biogas plants [27]. However, most of these products contain high levels of nitrogen and phosphorus (P), which is risky for the leaching of nitrate and phosphate. Many studies have shown that willows and poplars have high evapotranspiration rates and are able to uptake large amounts of nutrients present in waste, thus allowing significant wastewater disposal over the growing season [28]. Furthermore, some microorganisms, mainly bacteria, are capable of converting phosphates (through solubilization and mineralization processes), and in turn supplying P to plants [29]. Soil enzymes such as acid phosphatases and alkaline phosphatases aid in enzymatically mineralizing P hydrolytically [29]. These enzymes improve the P supply to plants and strengthen the activity of many beneficial microorganisms in the adjacent soil [29]. Additionally, studies have shown that phosphatases are involved in plant growth promotion, activity against plant pathogens, waste remediation and metal recovery [30–33].

The cultivation area of SRCs is expected to increase in many European countries, such as Sweden [27], Germany [34], Ireland [35] and England [25]. As the land under SRC cultivation increases, information on the potential impact of SRC on soil quality and nutrient use efficiency is needed. Early decreases in the nutrient availability under *Salix purpurea* (cv. Hotel) within the first cutting cycle were described from a Canadian site by Ens et al. [36].

Thus far, pure cultures of one *Salix* species are the common praxis, but mixed growth was tested during the last years [37]. Species mixtures can be more efficient in the nutrient mobilization [38], since they combine e.g., different microbial communities in the rhizosphere [39]. However, the impact of mixed growth on the nutrient availability was scarcely tested for *Salix* spp. thus far. We hypothesize that species mixtures of *Salix* can have a higher nutrient mobilization and soil nutrient availability than pure stands by their higher microbial diversity, and thereby, activity in the rhizosphere.

Therefore, the main aims of this study were: (i) to evaluate the impact of growth of *Salix* species/varieties in pure and mixed cultures on the nutrient availability in the soil at two test sites with different soil and climate conditions; and (ii) to analyze the effects of mixed vs. pure growth on soil enzymatic P mobilization by phosphatases.

2. Material and Methods

2.1. Study Sites and Soil Sampling

The SRCs selected for this study are among two of the three experimental field sites of the ECOLINK-*Salix* project. The goal of this project is to investigate the relationship between genotype diversity, genotype identity, productivity and ecosystem function [37]. The two SRC sites are located in Uppsala in Central Sweden (59°49′ N 17°39′ E) and Rostock in Northern Germany (54°02′ N 12°05′ E). These two field sites with different climatic and soil conditions were selected to test whether the effects of *Salix* species and mixture are common or limited to defined conditions only. The dominating soil type at the site in Uppsala is a Vertic Cambisol and was previously arable farmland. The area of 4147 m^2 is divided into 45 plots (92.16 m^2 in size). In 2014, 6480 trees of four different species/varieties were planted on this site. The species/variety pool of the trial in Uppsala includes four different *Salix* varieties partly belonging to different species [37], of which the pure and mixed culture for two of them was selected for the present study: *Salix dasyclados* var. 'Loden' (acronym L) and *S. schwerinii* × *viminalis* var. 'Tora' (T), based on their significant physiological differences [37]. The mean annual precipitation sum between March and October of 2014 to 2016 was 374 mm and the mean annual temperature between March and October of 2014 to 2016 was 11.0 °C in Uppsala. Samples from the trial in Uppsala were collected in an early stage (initial plantation year, 2014) and late plantation stage (after the first cutting cycle, 2018) of the plantation.

The site in Northern Germany near Rostock was previously used as arable farmland and is established on a Stagnic Cambisol. The area of 829 m^2 is smaller than in Uppsala, due to space and funding restrictions, which strongly compromised the trial size [37]. However, the plot size remained the same as in Uppsala, resulting in nine plots in Rostock. As a consequence of the smaller size of the trial area, the number of trees planted in 2014 was reduced to 1296, comprising only two species/varieties of *Salix* [Tora (T) and Loden (L)] instead of four [39,40]. The mean annual precipitation sum between March and October of 2014 to 2016 was lower in Rostock than Uppsala, with 281 mm, whereas the mean annual temperature between March and October of 2014 to 2016 was higher at 13.3 °C. Samples from the trial in Rostock were collected in an early stage (initial plantation year, 2014) and late plantation stage (after the first cutting cycle, 2018) of the plantation.

2.2. Planting Design and Sample Collection

In preparation for the experiment, both sites were treated with Roundup (glyphosate, 4 L ha^{-1}) in order to kill any existing weeds in the trial areas, which were subsequently cultivated with a rotavator prior to planting [37]. The planting of the 18 cm long stem cuttings was carried out manually [37]. All the cuttings were obtained from the same stock and were soaked in water for two days before being planted in such a way that the tips of the cuttings were flush with the surface [37]. In the beginning, the trial sites were weeded by hand; later, the weeds were controlled by mowing between the rows of plants when necessary [37]. No additional nutrient fertilizers were applied [37].

The planting set up on both the sites was a randomized block design with three replicates (blocks). The blocks in Uppsala have 15 plots each (i.e., four species/varieties and three replicates), whereas the blocks in Rostock consist of three plots [40]. The four (Uppsala) or two (Rostock) species/varieties of *Salix* were planted in every possible combination. Thus, some plots were planted with only one variety (e.g., L or T) pure cultures, some with mixtures of two varieties (e.g., LT) and, in Uppsala, even plots with three or four varieties were planted [37].

The patterns in which the cuttings were planted differed according to the number of species/varieties in the plots: if there were two species/varieties, they were planted in a checker board pattern; if there were three or four species/varieties, their planting positions were randomized, although with the single restriction that no two individuals of the same species/variety should be planted directly next to each other in one row [37]. Twelve rows of twelve plants were fit into the 9.6 m × 9.6 m plots with every other row being set off [37]. This led to a hexagonal planting pattern with 0.8 m between every plant.

The selected two *Salix* species/varieties display contrasting characteristics (Weih and Nordh, 2002). For example, *S. schwerinii* × *viminalis* var. 'Tora' (T) is generally high-performing but less stress resistant, while *S. dasyclados* var. 'Loden' (L) is sturdier and more stress-tolerant.

Soil samples were taken with a soil corer (3 cm diameter) down to 10 cm soil depth with five replicates per plot in spring 2014 and 2018. The early plantation stage was defined as the year of establishment of the short rotation coppice (initial, 2014). The late plantation stage was defined as the year after the first cutting cycle (after four years of growth, 2018).

This soil depth was selected, since the highest fine root density of *Salix* spp. was revealed in this range [41], and therefore, the highest soil ecological impacts were assumed at this depth. For the soil chemical analyses, soil was sieved <2 mm. Soil phosphatases were measured in fresh wet soil. All other soil chemical properties were measured in air-dried soil.

2.3. Biochemical Analyses of Soil

The activity of acid and alkaline phosphatases in the soil were determined colorimetrically according to [42]. The enzyme activities were expressed as µg p nitrophenol (pNP) g^{-1} soil h^{-1} released from the pre-given substrate solution (p-nitrophenyl-phosphate) within one hour of incubation in modified universal buffer with pH 6.5 (for acid phosphatases) and pH 11 (for alkaline phosphatases) in April 2014 and April 2018.

2.4. Chemical Analyses of Soil

The total carbon (TC) and total nitrogen (TN) concentrations of soil samples were determined by dry combustion using a VARIO EL analyzer (Vario EL Fa. Foss Heraeus, Hanau, Germany). The concentration of SOC was valued by deducting the separately determined inorganic C (dissolution with HCl and volumetric CO_2-determination) from the concentration of TC.

The soil pH was measured in a 0.01 M $CaCl_2$ solution using a soil:solution ratio of w/v 1/2.5. Double lactate-extractable P (P_{dl}), Mg (Mg_{dl}) and K (K_{dl}) were considered to be the plant-available P fractions (e.g., [43]) and were determined by extracting P, Mg and K from 12 g soil with 150 mL lactate solution ($C_6H_{10}CaO_6$ * H_2O + 10 N HCl) according to [44]. Concentrations of P, Mg and K were determined with inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 8300, Perkin Elmer, Waltham, MA, USA) at wavelengths of 214.914 nm, 285.213 nm and 766.490 nm, respectively.

2.5. Biomass Measurements

During the late winter of 2016/2017, i.e., three growing seasons after planting, here representing the "late plantation stage", all individual shoots within a central measurement area of 8.0 m × 3.2 m of each plot were cut at 0.1 m above ground and weighed in fresh condition (fresh weight). A stratified sample of 30 shoots per species/variety was done among the plants situated outside the central measurement area of all pure culture plots to determine the relationships between fresh and dry weights of shoots separately for all species/varieties. The dry weights (biomasses) of the stratified sample shoots were determined after oven-drying at 70 °C for 96 h, and the species/variety specific regressions between the fresh and dry weights of the stratified samples were used to estimate the biomasses of all individual shoots sampled within the central measurement area of all plots [37].

2.6. Statistical Analyses

The effect of the site, the growth design and their interactions on the soil properties were analyzed by two-way ANOVA using the software PAST [45]. Statistical analysis was performed using the Statistica software package (version 13.0, StatSoft, Tulsa). Principal Component Analysis (PCA) was performed using R package. The samples used in the PCA were attributes (mean values) measured in two test sites (Rostock and Uppsala), two plantation stages (early and late) and three species identity and culture conditions ('Loden' pure culture, 'Tora' pure culture and a mixture of 'Loden' and 'Tora'). The attributes analyzed in the present study were: alkaline phosphatase activity, acidic phosphatase activity, willow biomass and plant-available K, Mg and P content in the soil.

3. Results

3.1. Plant-Available Nutrient Contents (K, Mg and P) in the Soil

The initial plant-available concentrations of K and Mg in the soil differed significantly between the two test sites and between the early and late plantation stage per test site (Table 1). The plant-available concentration of P was low and at the same level at both test sites (Figure 1). The plant-available concentrations of P and K in soil significantly decreased with the progressing willow growth (initial vs. 4 years of growth) at the test site Rostock (Figure 1 and Table 1).

Table 1. Nutrient contents (K, Mg and P) in mg/100 g soil in in sampling sites (a) Rostock and (b) Uppsala initally and after 4 years for each species identity and cultivation condition (Loden, Tora and their mixture). The biomass of the willow species was measured after harvest (kg dry matter per plot).

(a) Rostock							
Species	**Initial**			**After 4 Years**			**Biomass (First Harvest)**
	K (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	(kg dry matter/plot)
Loden [L]	13.4 ± 6.1	25.2 ± 9.5	6.8 ± 1.6 ^{A}	10 ± 2.2	20.4 ± 3.8	4.4 ± 0.01 ^{B}	16.14 ± 4.94
Tora [T]	32.9 ± 10.7 ^{A}	27.5 ± 5.9	5.8 ± 0.9 ^{A}	10.7 ± 3 ^{B}	22.0 ± 0.3	4 ± 0.1 ^{B}	30.71 ± 11.94
Loden, Tora [LT]	23.5 ± 14.3	21.4 ± 9.4	5.2 ± 1.9	10.2 ± 3.6	21.1 ± 4.3	4.4 ± 1.1	19.57 ± 5.21
(b) Uppsala							
Species	**Initial**			**After 4 Years**			**Biomass (First Harvest)**
	K (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	P (mg/100 g)	(kg dry matter/plot)
Loden [L]	17.6 ± 3.3	25.9 ± 1.6 ^{B}	5.1 ± 0.3 ^{A}	22.2 ± 2 *	32.1 ± 1.8 ^{A*}	4.3 ± 0.2 ^{B}	11.19 ± 5.14
Tora [T]	16.6 ± 2	27.7 ± 3.3	4.5 ± 0.6	19.9 ± 3.6 *	28.8 ± 3.3 *	3.6 ± 0.4	13.67 ± 2.89
Loden, Tora [LT]	17.8 ± 4.1	30.8 ± 12.3	6.0 ± 2.4	23.6 ± 4.6 *	27.9 ± 6.9	5.0 ± 1.8	13.76 ± 4.74

Soil properties were compared by site, growth stages (early and late plantation stage) and species (plots: L—Loden, T—Tora and LT—mixture). Values are means ± SDs (n = 3). The significant differences with $p < 0.05$ are marked by the following symbols: *—differences between sites, small letters—differences between species within one site, capital letters—differences between growth stages within one site.

Conversely, the amount of plant-available Mg in Uppsala soils increased from early to late plantation stages mainly under Loden (Figure 1). The plant-available concentration of K at the test site Uppsala was significantly higher than at the test site Rostock (see Tables 1 and 2). Likewise, the plant-available concentration of Mg in soil was higher in pure culture (Loden and Tora) in Uppsala in comparison to Rostock after 4 years of growth.

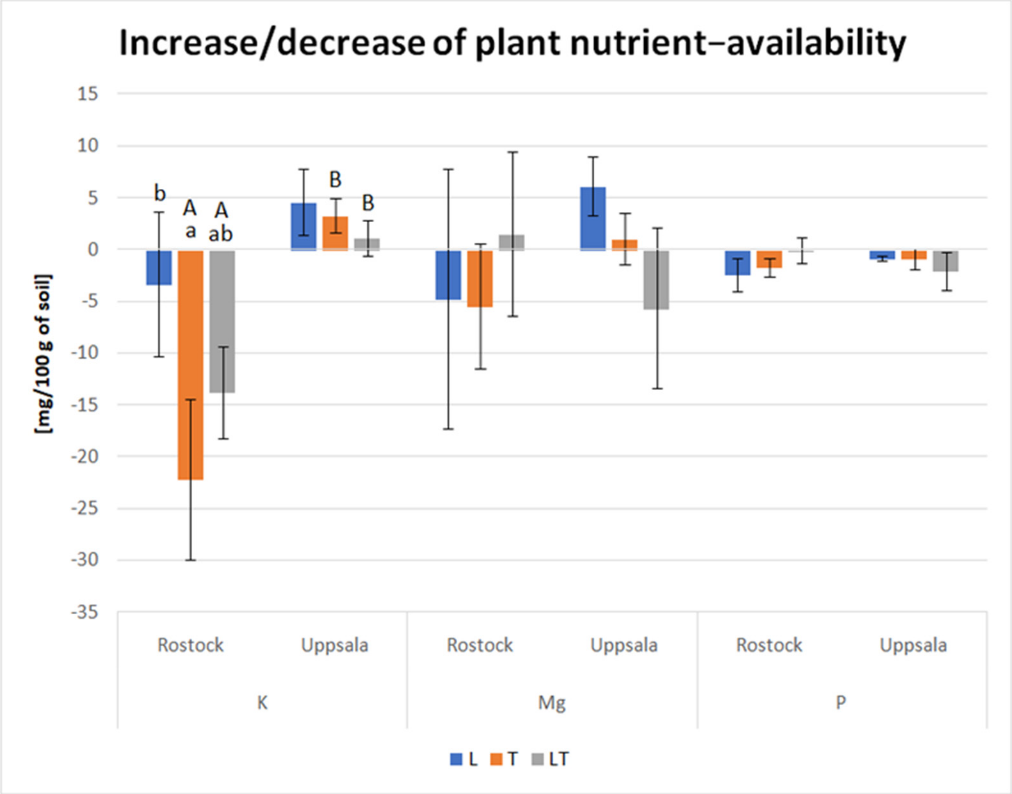

Figure 1. Nutrient levels increase/decrease between the years 2014 and 2018 in the soil at the two sites (Rostock and Uppsala). Small letters represent significant differences between the *Salix* species or the growth design on the same sampling site and capital letters represent the significant difference between sampling sites.

Table 2. Results of the two-way analysis of variance (ANOVA) on the effect of site, the growth design (with different host plant diversity; pure vs. mixture) and their interactions (site × growth design) on soil properties under *Salix* in spring 2014 (initial) and spring 2018 (after 4 years of growth).

Parameter		**Site**		**Growth Design**		**Site × Growth Design**	
		Initial	**4 Years**	**Initial**	**4 Years**	**Initial**	**4 Years**
Plant-available P content	p	0.304	0.950	0.603	0.249	0.335	0.590
	F	1.15	0.004	0.53	1.56	1.20	0.335
Plant-available Mg content	p	0.092	<0.001	0.612	0.747	0.101	0.495
	F	3.36	20.48	0.51	0.29	2.79	0.74
Plant-available K content	p	0.020	<0.001	0.053	0.702	0.039	0.536
	F	7.15	606.01	3.79	3.94	4.28	7.13
Alkaline phosphatase activity	p	0.378	<0.001	0.928	0.065	0.344	0.184
	F	0.84	95.30	0.07	3.45	1.17	1.95
Acid phosphatase activity	p	0.200	<0.001	0.016	0.513	0.147	0.297
	F	1.84	198.20	5.92	0.71	2.26	1.34

3.2. *Acid and Alkaline Phosphatase Activity in the Soil*

The site and the growth design affected the activities of acid and alkaline phosphatases significantly (Table 2). The alkaline phosphatase activity in the soil Rostock was significantly higher at the early plantation stages, and a significance between species (mainly in Loden) was observed (Figure 2). In the late plantation stage, the soils showed very low activity, specifically in the soil under Loden and under the mixture. Initially, under Loden, significantly higher alkaline phosphatase activity in the soil was revealed at the test site Rostock (Figure 2), while after 4 years under Tora and Loden, significantly higher activities were measured than under the mixture at the test site Uppsala.

The activity of acid phosphatases significantly increased in the early plantation stages of willow species than in the late plantation stages in Rostock (Figure 2). The highest activity in soil was observed in plots with the mixed culture plots (LT) in comparison to the plots with monocultures Tora and Loden. No significant differences were observed among the monocultures and mixed culture plots in the early nor in the late plantation stages in Uppsala. Only plots with Loden displayed a statistical significance, with the highest activity in the late plantation stage. Overall, the acid phosphatase activity was the lowest at the late plantation stages of species in Rostock. On average, the activity in the early plantation stage was approximately 320 µg p-nitrophenol g^{-1} DM h^{-1}, whereas in the late plantation stage, it lowered to an average of approximately 130 µg p-nitrophenol g^{-1} DM h^{-1}. A significantly high phosphatase activity was seen in the late plantation stages of willow species in Uppsala. Similarly, this activity was the lowest in Rostock.

Overall, a significantly higher acid phosphatase production was recorded at both the investigated sites compared to alkaline phosphatase production (Figure 2).

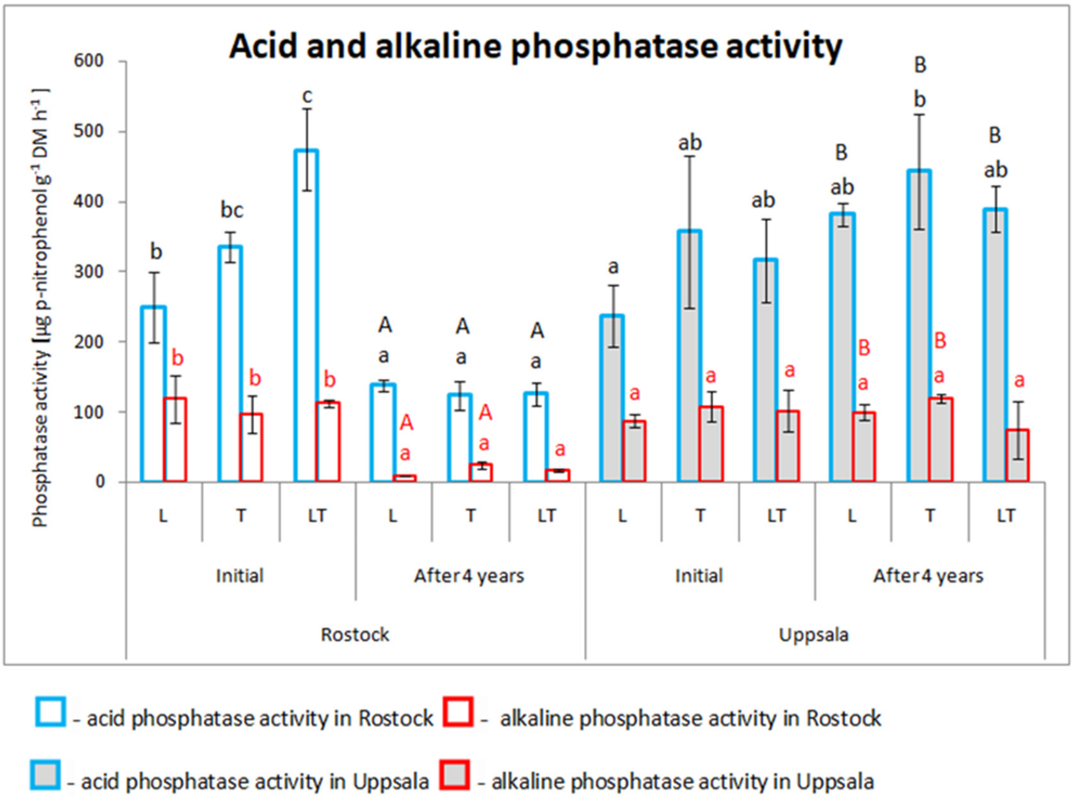

Figure 2. The alkaline phosphatase and acid phosphatase activity [µg p-nitrophenol g^{-1} DM h^{-1}] in soils obtained from Rostock and Uppsala. The data present comparisons between two sites, Rostock (on the left site) and Uppsala (on the right), between growth stages (initial and after 4 years) and species (plots: L—Loden, T—Tora and LT—mixture). Values are means ± SDs (n = 3). The significant differences with $p < 0.05$ are marked by the following symbols: small letters—differences between species within one site, capital letters—differences between initial and after four years. DM—dry matter.

Regardless of the tested parameters, approximately 55% of the total variance was explained by the first two components in the PCA analysis (Figure 3). The PCA analysis revealed that the samples were differentiated mainly based on the test sites, i.e., Rostock and Uppsala. A positive tendency towards the increase in willow biomass production was observed for Rostock samples with higher acid and alkaline phosphatase activity.

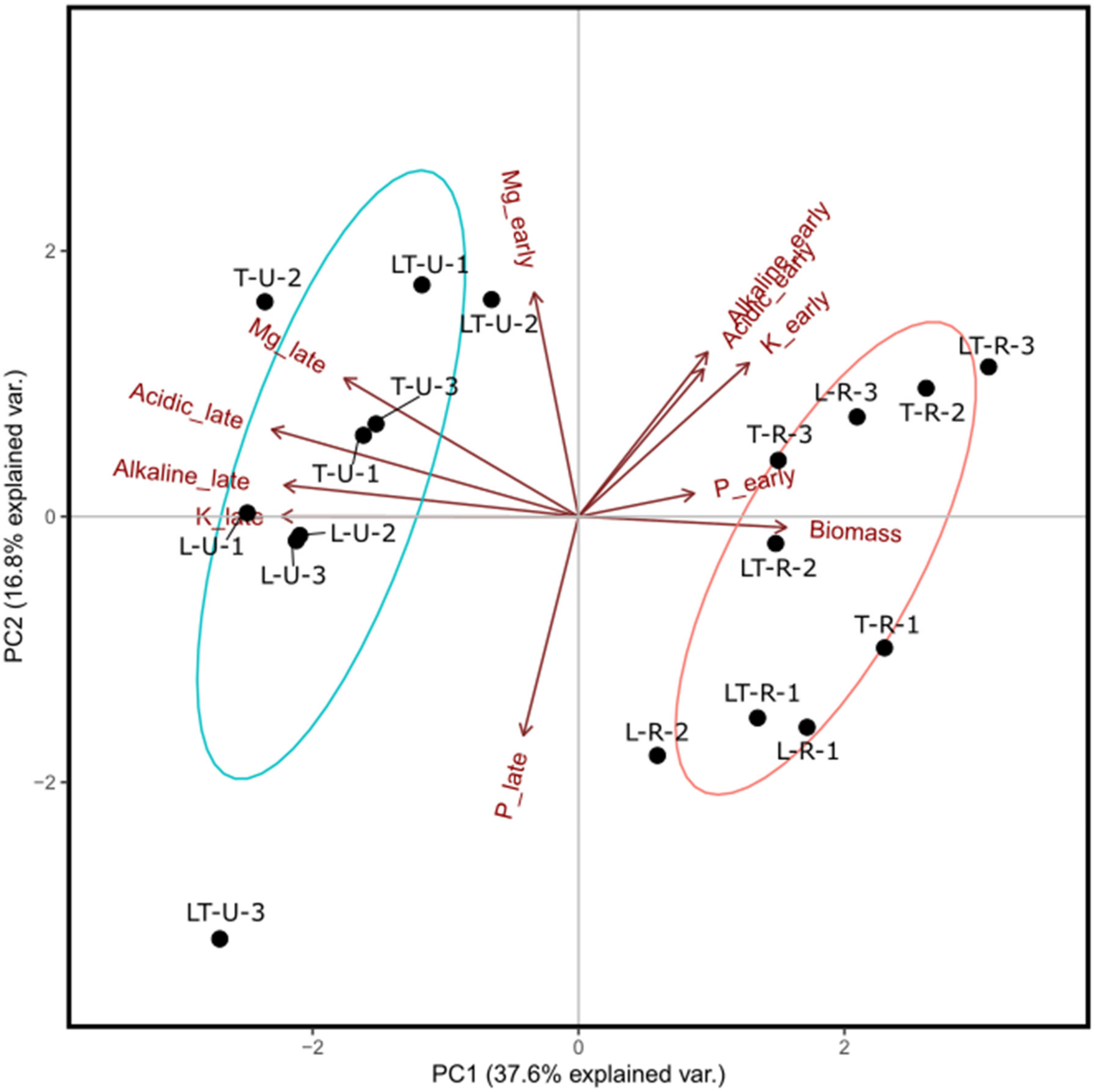

Figure 3. Grouping of samples and corresponding variable component loadings according to the Principal Component Analysis (PCA). Samples are attributes (mean values of the attributes indicated by arrows) measured in two test sites (Rostock—R and Uppsala—U), two plantation stages (initial (early) and after 4 years (late)) and three species identity and culture conditions (L—Loden pure culture, T—Tora pure culture and LT—mixture of Loden and Tora). Attributes were soil phosphatase activity (Alkaline and Acid), willow biomass and soil nutrient content (K, Mg and P in early and late plantation stages). PC1 explained 37.6% of total variance; PC2 explained 18.8% of total variance.

4. Discussion

Short rotation coppices (SRCs) generate crops used in renewable energy generation in Europe. The success of SRC establishment can be assessed by studying the adaptability of crops by monitoring their growth, climate and site conditions. Factors such as climate, soil nutrient availability, plant species and growth design may significantly influence plant nutrient cycling and overall biomass production. This study investigated the Ecolink *SALIX* SRC plantations located in Germany (Rostock) and Sweden (Uppsala). Both test sites were maintained in a similar way and planted with same two *Salix* species and their mixture. We analyzed the effect of the growth design on the plant availability and hydrolytic mobilization of P at the year of planting and after 4 years of growth, including the first harvest. The test cultivars Loden and Tora were selected for our experiment because they are both phenotypically and genotypically very distinct *Salix* species. Loden is rather slow-growing but more stress-tolerant, while Tora is generally high-performing and less stress-tolerant [37].

The initial concentrations of plant-available nutrients (Mg, K and P) at both test sites in the present study were high compared to the recommended level for arable crops [44] for Mg and K, but below the recommended level (10–18 mg P/100 g soil) for an optimal P supply. The plant-available concentration of Mg in the soils of Rostock and Uppsala was even higher than the recommended value of >19, i.e., to 32 mg/100 g soil for an optimal plant supply [44]. Moreover, the soil of the test site Rostock displayed high initial levels of plant-available K concentrations (see Table 1).

Overall, the biomass production at the test site Rostock was higher than at the test site Uppsala; however, no significant differences were observed between species and pure vs. mixed culture plots [37]. The biomass production was not correlated with the plant-availability of one of the tested nutrients (Mg, K, P); however, a correlation between the phosphatase activities and the biomass production was indicated (see Figure 3).

In agreement with Ens et al. [36], we measured significantly decreased plant-available P concentrations in the soil under the pure stands after the first cutting period, and a significant site effect on the P cycling (phosphatase activities, see Table 2) was observed. However, significant differences were observed for soil nutrient concentrations (mainly P and K) between the initial stage early and after four years of growth at both test sites (see Table 1). The acid and alkaline phosphatase activities decreased strongly from the initial level to the 4-year growth stage, which might be caused by the former grassland vegetation with a higher fine root density [46].

A general effect of the species and the growth design on the phosphatase activity under *Salix* is in agreement with the results of the mycorrhizosphere observation by Baum et al. [47]. However, only two *Salix* species were investigated in the present study due to the limitation of available plant variants at the test site Rostock in Germany (only Loden and Tora were present). Increased number of plant species and a higher amount of diversity in the mixtures (three or more species) might have increased the validity of the present information. Furthermore, a joint impact of the P and N supply might be assumed [36] and was not investigated in the present study.

The initial nutrient surplus at the arable test sites in Sweden and Germany agrees with results of former investigations of SRC [48] and underlines no need for fertilization in the first cutting period at such sites. This is because formerly arable farmland was usually regularly fertilized, which often results in high nutrient contents [49]. The nutrient concentrations in the soil changed significantly within the first four years (see Table 1). The cultivar Loden, which was included in the present study, is frequently reported with great potential in nutrient acquisition from soil, especially nitrogen and increased biomass production when paired with other *Salix* species [40]. The analysis of the P content in soil of both the sampling sites showed a significant decrease from early plantation to late plantation stage, which might suggest that Loden is efficient in P uptake. Since both experimental sites were not fertilized after willows were planted, most of the P present is in organic or low soluble form, which is not easily accessible to plants. P depletion in Uppsala and Rostock was also paired with a significant increase and decrease of acid phosphatase activity, respectively (see Figures 1 and 2). This inconsistency might suggest that acid phosphatase activity is not strongly connected with P supply in soil, but may be connected with other factors. Study performed by Criquet et al. [50] revealed that increased leaf litter moisture is positively correlated with acid phosphatase activity. Additionally, experiments performed in three forest ecosystems in China showed that increased precipitation during the dry season had a positive effect on enzyme activity [51]. The cultivar Tora showed slightly fewer prominent differences from the initial nutrient availability to the level after the first cutting cycle; only in Rostock site it differed in both P and Mg between these two sampling dates. Although P depletion was not as severe as for Loden, the plant-available Mg content decreased by almost two-fold compared to the initial content.

Of note, differences were observed in willow biomass production at the two test sites, which may be due to the varying nutrient concentrations in soils. Most of the plant-available nutrient concentrations (Mg and P) were decreased in the soil within the first cutting period, although the level of K varied at both the test site and growth stages. The PCA analysis revealed that biomass was positively correlated with P and K during the early plantation stage and negatively with late plantation stage concentration of P and K (Figure 3). The correlation was prominent, although we observed K depletion in one experimental site and an increase in the other site. Willows are known to efficiently uptake organic P when paired with ectomycorrhizal fungi [52]. This suggests that the presence of these two compounds during early plantation stages is key to reaching higher biomass production efficiency in later plantation stages. Additionally, the K content was in direct correlation with alkaline and acid phosphatase activity in the early and late plantation stages, respectively, thus indicating that changes in phosphatase activity are not bound to soil P concentration but to other, more complex sets of factors. High correlation of plant-available K with phosphatase activity is probably connected with its important role as a co-factor of many enzymes. Tabaldi et al. [53] investigated the effect of various metals on *Cucumis sativus* L., e.g., Zn, K and Na. As a result, they observed increased acid phosphatase activity in higher presence of K ions.

Acid phosphatase activity was about three-fold higher than alkaline phosphatase. According to measurements performed in our previous study, pH on both sampling site is around 6 (measurements done at 2018 and 2019) [39], which promotes the activity of acid phosphatases. pH in which acid phosphatase is active is between 4.5–6, whereas for alkaline, this is 8–11 [54]. Additionally, the pH value in SRCs is known to drop slowly with time, which further promotes the activity of acid over alkaline phosphatase [55]. Another very important factor in acid phosphatase activity is the presence of arbuscular and ectomycorrhizal fungi. Baum et al. [47] pointed out the impact of both mycorrhiza types on various factors, including acid phosphatase activity. Loden was mostly colonized by ectomycorrhizal fungi and showed higher acid phosphatase activity in pure cultures, while Tora was colonized by arbuscular mycorrhizal fungi with slightly lower activity. Additionally, they reported that mixed growth of *Salix* possessed higher phosphatase activity than monocultures [47]. The abovementioned factors contribute to increased P mobilization and were in agreement with the insignificant decrease of the plant-available P concentrations in the soil under mixed growth of *Salix* species within the first cutting cycle in the present study.

5. Conclusions

The site impacts are the main controls of the changes in the concentrations of plant-available nutrients under *Salix*. The changes of the plant availability of P within one cutting cycle are generally low, independently of the site and growth design. Mixed growth of *Salix* species promotes the activity of alkaline phosphatases in P-deficient soil conditions. The impact of the growth design on the nutrient cycling differs significantly and site-specifically in the direction and amplitude. The future challenge will be to select a site-specific optimized growth design.

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