Dependence of cluster (dauciform) root formation in Cyperaceae on iron, phosphorus and water availability and implications for species distribution

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Abstract

Some Cyperaceae species have the capacity to form dauciform roots. It has been shown that these root structures are built as a response to internal phosphorus deficiency. Whether other factors also influence dauciform root formation is unknown. Also unknown is, what implications the ability to built dauciform roots has on species distribution in the field. The aim of this study was to investigate whether dauciform root formation depends on iron and water availability and if dauciform root-bearing Cyperaceae fill a special ecological niche.

In a pot experiment, dauciform root development was investigated in two sedge species (*Carex panicea* and *Schoenus nigricans*) grown at three different phosphorus, three iron, and two water levels (medium and high). After 12 weeks of supply, dauciform roots were counted and biomass was weighted. In a field survey, eight mires distributed over northern Switzerland were sampled. Plant species composition was recorded in ten plots of 2x2 m^2 per mire; aboveground plant material of Cyperaceae species was collected in every plot and analyzed for Fe, P and N content. Nutrient contents were used to estimate nutrient availability of each plot.

Dauciform root formation in the pot experiment did depend on phosphorus supply, however, not on iron or water availability. Likewise, all biomass fractions were independent of iron and water, but not of phosphorus supply. This indicates that overall, phosphorus seems to be of higher importance than iron and water availability.

Cyperaceae with the ability to form dauciform roots occurred in all vegetation types which had been investigated. The presence of these species was neither associated with phosphorus, nor iron or water availability. This indicates that dauciform root-bearing Cyperaceae do not have a special ecological niche. However, in only 14 out of 80 plots species building dauciform roots could be found, and these plots were the less acid ones investigated. Thus, it is likely that dauciform root-bearing Cyperaceae mainly occur in mires with higher pH than the ones sampled in this study. To get a deeper knowledge about the distribution of dauciform root forming species, less acid mires should be investigated.

Introduction

Cluster roots are brush-like root structures, which are built within 10 days and accumulate carboxylates and phosphatase (Lambers and Colmer 2005, Shane et al. 2006). Then, they die off and release their substances in an exudative burst (Playsted et al. 2006, Shane et al. 2006). The released compounds solubilize minerals and organic nutrients, thus enhance nutrient, especially phosphorus (P) availability (Playsted et al. 2006, Shane et al. 2006).

Cluster roots have only been found in a few families; the major ones are Proteaceae, Fabaceae, Restionaceae and Cyperaceae (Lambers et al. 2006). As cluster roots considerably differ in structure and morphology among families, these root structures are termed differently for most families: proteoid roots for Proteaceae, capillaroid roots for Restionaceae, and dauciform roots for Cyperaceae (Lambers et al. 2006). Despite differing in structure and morphology, cluster roots do not seem to differ in function (Dinkelaker et al. 1995, Shane et al. 2006). Most families in which cluster-bearing species occur are distributed in a restricted area. Proteaceae, for instance, a family in which cluster roots are universally found, only occur in Australia and South Africa, and Restionaceae are restricted to the southern hemisphere (Lamont 2003). In contrast, Cyperaceae is a cosmopolitan family (Ball 1990), and it is the only one found to produce cluster roots in Europe. Up to the present, dauciform roots have been found in 27 species, nearly half of the Cyperaceae species searched for such root structures (Shane et al. 2005). Astonishingly, even closely related Cyperaceae species may differ in their ability to form dauciform roots (Sabine Güsewell, ETH Zurich, personal communication). Cyperaceae species mostly are ecologically specialized and mainly occur in nutrient-poor ecosystems (Grime 2001).

It is widely acknowledged that dauciform roots of Cyperaceae, like most cluster roots, are built as a response to internal phosphorus deficiency, and are believed to function as a mechanism to increase P availability (e.g. Bakker et al. 2005, Lambers and Colmer 2005, Playsted et al. 2006). Phosphorus, however, is the only factor, which has been closely examined for a possible effect on dauciform root formation so far; though other factors, namely iron (Fe) and water availability also seem to influence cluster root production in other families. Iron deficiency, similarly to phosphorus deficiency, has been found to promote cluster root formation in a number of species belonging to different families, for instance in *Casuarina glauca* (Rosenfield et al. 1991, Arahou and Diem 1997), *Ficus benjamina* L. (Rosenfield et al. 1991), *Lupinus albus* (Gardner et al. 1982) and *L. consentinii* (White and Robson 1989). That iron availability may be enhanced by cluster roots exudations is not surprising when considering that mechanisms for iron acquisition include the acidification of the soil (Marschner et al. 1986, Garrido et al. 2006), an action which is also enhanced by cluster root exudates. However, to my knowledge it has not been investigated yet whether dauciform root production is also promoted by iron deficiency in Cyperaceae. Also hardly investigated is whether water availability influences dauciform root formation. Davies (1970, cited in Davies et al. 1973) observed a low number of dauciform roots in *Carex flacca* and *C. panicea* in very dry habitats; but whether this pattern is also valid for different water availabilities under wetter conditions, conditions under which many Cyperaceae species occur, is unknown. The investigation of the dependence of dauciform root formation on iron, water and phosphorus availability is of special interest, as these factors highly interact with each other: Not reduced iron binds phosphorus, and flooding leads to reducing conditions which in turn lead to the release of both phosphate and reduced iron due to anaerobic processes (Bakker et al. 2006, Loeb et al. 2008, Banach et al. 2009). Due to these interactions it is difficult to separate these effects from each other, and I am not aware of any previous study, which tried to investigate whether dauciform root formation is not only influenced by phosphorus supply, but also by iron and water availability and their interactions.

When studying dauciform root formation an important question is how these root structures influence species distribution in the field. To my knowledge only one study tried to find the niche of species forming cluster roots. Lambers et al. (2006) investigated the occurrence of cluster-bearing Proteaceae and found that in south-western Western Australia they predominate soils which are most phosphorus impoverished. If the distribution of dauciform roots producing Cyperaceae depends on soil P level is unknown (Lambers et al. 2006). However, this is of special interest, as some European Cyperaceae, namely *Carex panicea* and *Schoenus nigricans*, seem to develop only small cluster roots of approximately 2-4 mm length and 1-2 mm width (personal observation). This was also found for other European *Carex* species (Sabine Güsewell, ETH Zurich, personal communication). In contrast, cluster roots of Proteaceae are much larger; they range 2-75 mm in length and 1-34 mm in width (Lamont 1983). Therefore, European Cyperaceae species, which are capable of forming dauciform roots, may not respond in the same way as Proteaceae to different environmental conditions in the field. Nevertheless, dauciform roots are, as proteoid roots, built in response to phosphorus deficiency (Playsted et al. 2006). Thus, it seems likely that cluster-bearing Cyperaceae also are advantaged under phosphorus poor conditions.

My study is devoted to the following questions: (1) does dauciform root formation depend on Fe and water availability? (2) Are these effects influenced by phosphorus supply? (3) Do species producing dauciform roots fill a special ecological niche (e.g. sites with low P and Fe availability)? I hypothesize that dauciform root formation in wetland sedges is promoted by low iron and high water availability, and that these effects are mostly pronounced at intermediate P-level. Further, I expect that species forming dauciform roots occur at phosphorus and iron poorest sites and at sites with high water table.

Materials and methods

Pot experiment

Plant cultivation

Two Cyperaceae species which are known to built dauciform roots were selected for the experiment: *Carex panicea* and *Schoenus nigricans* (Shane et al. 2005). The aim was to investigate the reaction in dauciform root formation in Cyperaceae, not to make a statement about the variability of a species. Nevertheless, to include some variability, plants of three populations of *C. panicea* were collected; only one population of *S. nigricans* was included. Plant collection occurred during the period May to June 2009; from a mire at Neuenburgersee next to Cudrefin (both species) and Greifensee between Greifensee and Schwerzenbach (two populations of *C. panicea*). At all three sites of population collection, plants were taken within approximately 20 m^2. It was intended to represent the gene pool of the population. Thus, plants were individually taken and not split into clones. Plants were carefully dug out and they were kept on tap water until new roots had formed (2.5-5 weeks). Then, each plant was planted into a 1 litre pot with quartz sand (0.1-0.6 mm grain size). In the first weeks of the experiment, the initial aboveground biomass died and only newly formed biomass was included in the measurement of the living aboveground biomass. The second Greifensee-population of *C. panicea* was planted 16 days later in order to replace a population, which did not form any new roots in tap water.

Two weeks before starting with the treatment, all pots were transferred to a greenhouse (day/night regime: 16/8 h, light: 480-500 Wm^{-2}, temperature regime: 25/15ºC) and regularly irrigated with deionised water. Pots were placed on trays, which were shifted regularly on the greenhouse bench to compensate for heterogeneity. Pots were also shifted within trays.

The experiment started in the middle of July and lasted for 12 weeks. Thirteen treatments were applied, combining three phosphorus supplies with three iron supplies and two water levels. The design was not full factorial since the high water level was only combined with medium and high P- and Fe-supplies (Table 1). All other essential elements were supplied in constant, non-limiting amounts. Each treatment had four replicates. To simulate high and low water level, cachepots were used in which three holes had been bored (diameter of bore: 5 mm) at 2.5 and 11.5 cm for low and high water level, respectively.

Nutrient treatments were supplied once a week. To avoid nutrient interactions, P and Fe were supplied at different days in the week with at least two days of time lag: During the first half of the week, phosphorus, nitrogen and potassium were supplied together with a pipette; in the second half, calcium, magnesium and micronutrients (diluted up to a supply of 30 ml per plant to avoid chemical precipitation) and iron (with a pipette) were supplied. To account for the increasing size of the plants, 2/3 of the total amount of nutrients was supplied after the 6th week. Plants were watered every 1-3 days with deionised water, omitting the day after having applied a nutrient treatment.

Harvest and analyses

From 19th- 30th October 09, all replicates of the pot experiment were harvested in sequence. First, aboveground plant biomass was clipped close to the sand surface and sorted into living and dead parts. Then, roots were washed out gently from the sand and the number of mature cluster roots was determined on the root system. Branching of the root system was classified for three replicates according to Fitter (1982) as low (2 orders, i.e. herringbone root system), medium (3 orders) or high (more than 3 orders). Subsequently, roots were separated from the rhizome or the tussock basis. Finally, all biomass fractions were dried at 70ºC for at least 48h and weighted.

Eleven plants were not harvested, as they died during the first half of the experiment.

Table 1: Total amounts of Fe (supplied as Fe-EDTA), P (supplied as KH_2PO_2) and other elements supplied to each plant.

Treatment	P (mg)	Fe (μg)	Water level	N:P
P1 Fe1	1	80	low	45
P1 Fe2	1	240	low	45
P1 Fe3	1	720	low	45
P2 Fe1	3	80	low	15
P2 Fe2	3	240	low	15
P2 Fe3	3	720	low	15
P3 Fe1	9	80	low	5
P3 Fe2	9	240	low	5
P3 Fe3	9	720	low	5
P2 Fe2	3	240	high	15
P2 Fe3	3	720	high	15
P3 Fe2	9	240	high	5
P3 Fe3	9	720	high	5
Other elements (mg), all treatments				
N, K (KNO3)	45.000			
Ca (CaCl2*2H2O)	15.600			
Mg, S (MgSO4*7H2O)	4.800			
Cu (CuSO4*5H2O)	0.009			
B (H3BO3)	0.150			
Mn (MnCl2*4H2O)	0.072			
Mo (Na2MoO4*2H2O)	0.021			
Zn (ZnSO4)	0.036			

Vegetation survey

Study area and field data sampling

Eight mires distributed over the north of the Swiss Alps were selected for this study (Table 2). All mires except Salwidili (Sörenberg, Luzern) belonged to the federal moorland inventory of peatlands and transitional moors, thus were protected by national law. These mires were chosen because they were nearly undisturbed, had little nutrient input and covered a wide range of geological parameters. The long-term average annual precipitation range was 1500-2400 mm; the mean temperature was 10-15ºC and minus 2-5ºC in July and January, respectively (MeteoSchweiz: www.meteoschweiz.ch 2009). Most soils were acid peat soils; only the mire Hobacher did not contain any peat and the acidity was less pronounced there and at Schwendiseen.

Plant species composition was recorded in 10 plots of 2x2 m^2 per mire. Plots were arranged on 1-3 transects per mire, placed in order to cover all vegetation types, at 10-20 m intervals, depending on size of the mire. If a plot would have been disturbed by ditches, ponds, footprints or local nutrient rich conditions it was shifted by 1 meter to obtain undisturbed conditions. From July to August 2009, plant species composition was recorded and percentage cover of each species was visually estimated. Likewise, total cover of herbs, shrubs, trees and mosses was estimated. In each plot, aboveground plant material of approximately 10 plants per Cyperaceae species was collected for nutrient analysis. For determination of peat depth and for pH analysis, three soil samples were taken from 0-20 cm below the soil surface with a 5 cm diameter corer. In order to stop pH from changing, samples were frozen until pH analysis. For each plot, average ecological indicator values were calculated (Landolt in press).

Table 2: Study sites

Mire	Municipality (Canton)	Coordinates (Swiss grid)	Altitude	Core zone area (ha)	Geology
Hobacher	Oberiberg (Schwyz)	699.850 / 209.675	1350	8.2	fluvioacustric depositions[1]
Hinter Höhi/Bönisriet/ Stöcklerriet	Amden (St. Gallen)	731.000 / 226.200	1420	30.7	peat, below: chalk-flysch[2]
Mettilimoos	Entlebuch (Luzern)	649.725 / 203.750	1020	15.6	moraine[3]
Salwidili	Sörenberg (Luzern)	642.350 / 184.875	1330	0.5	fluvioacustric depositions[3]
Schwendiseen	Alt St. Johann (St. Gallen)	743.375 / 227.900	1160	1.4	peat, below: moraine[4]
Etang de la Gruère	Le Bémont, Montaucon, Saignelégier (Jura)	570.525 / 232.150	1000	21.5	marl[5]
La Tourbière de la Chaux-des-Breuleux	La Chaux des Breuleux, Saignelégier (Jura)	570.350 / 230.500	975	12.4	marl lime[5]
La Tourbière au sud des Veaux	Les Genevez (Jura)	574.000 / 232.375	1012	7.2	marl lime[5]

[1]Quereau (1893), [2]Herb and Franks-Dollfus (2003), [3]Institut für Geologie der Universität Bern and Sektion Geologischer Landesaufnahme des Bundesamtese für Wasser und Geologie (2005), [4]Heim and Oberholzer (1903), [5]Schlumpf (1901).

Plant and soil analyses

Plant material was dried at 70ºC for at least 48 hours and crushed. To determine the iron content of the plant material a sub-sample of known weight (0.1-0.3 mg) was acid digested in 5 ml of 30% HNO_3 (ultrapur) at 90ºC for a minimum of 8 h. Then, the digest was filtered and diluted up to 20 ml. The Fe concentration in the digest was determined using an AAS 240 FS atomic absorption spectrometer (Varian, Australia). To determine the amount of nitrogen and phosphorus, a second sub-sample was digested with the Kjeldahl method. Both Kjeldahl nitrogen and total phosphorus were determined using a continuous flow injection analyser (FIAStar, Foss Tecator, Höganäs, Sweden). If only one analysis could be done with the available plant biomass, the amount of Fe was determined. Nutrient contents were not analysed for plants with very low biomasses (e.g. *Carex pilulifera*).

For soil pH analysis 2.5 parts of 0.01 M $CaCl_2$ solution were added to 1 part of soil and mixed thoroughly. After two hours, the solutions were mixed again and pH was determined using a pH-meter (744 Metrohm).

Data analysis

All statistical analyses were performed with R 2.9.2.

Pot experiment

To study if iron, phosphorus and water supplies had a significant effect on dauciform root formation and biomasses, ANOVAs were fitted. As the design of the pot experiment was not full factorial, the effect of water levels and the three phosphorus levels had to be analysed separately, using three- and four-way ANOVAs, respectively. In both models, only two-way interactions were included as the higher interactions were not significant and the models with three- or four-way interactions yielded similar results to the models with two-way interactions. If necessary, data was log- or square root-transformed or outliers were removed to yield normal distribution. Pairs of groups were compared using Tukey's HSD test.

To test whether root branching differed among P, Fe, water level, species and sites a logistic model for binary response was used.

Vegetation survey

The recorded Cyperaceae species were allocated to the categories "species with the capacity to build dauciform roots" and "species without the capacity to build dauciform roots" according to

Table 3. To study differences in nutrient content between these groups, a Wilcoxon test was conducted for Fe, P and N content and N/P ratio. In order to weight the different species equally, the mean species values were used for this test.

Plant nutrient contents seem to reflect the nutrient availability of the soil (Wassen et al. 1995, Van Duren and Pegtel 2000). Therefore, in this study, iron and phosphorus availability of each plot was estimated through the nutrient contents of Cyperaceae species. Furthermore, species-specific differences were considered: The difference to the mean species value of ever species in a plot was calculated. For all plots, the average of these values of all species occurring in this plot was calculated. Then, this value was added to the average nutrient content of all species. This calculation method was used to estimate the iron, phosphorus and nitrogen content and N/P ratio of all plots. For all statistic analyses, this measure yielded closely similar statistic results to the mean nutrient value per plot derived from a two-way ANOVA for site and species. Species sampled in one mire only (i.e. *Carex pallescens* and *Trichophorum alpinum*) and species with uncertain determination (i.e. *Carex cf. pilulifera*) were not included in the calculation. *Carex cf. rostrata* was treated as *C. rostrata*.

The identification of variables best explaining nutrient contents of plots was conducted with a stepwise regression based on Akaike's information criterion AIC. The influence of iron and phosphorus availability on the number of species forming dauciform roots in a plot was analysed using poisson regression. The effects of nutrient availability, indicator values, mean pH and mean peat depth of each plot on the presence or absence of species forming dauciform roots was calculated with a logistic model for binary response (Chisq test).

To study whether dauciform root forming species exhibit a special ecological niche, a principal coordinates analysis was run on the vegetation matrix (distance method: bray-curtis). In this vegetation matrix, only species occurring in more than 3 plots were included, and percentage cover was log-transformed in order to diminish the influence of species with high covers on the result.

Table 3: Allocation of Cyperaceae species to the groups "species with the capacity to build dauciform roots" and "species without the capacity to build dauciform roots". Species, for which it is unknown whether or not they are able to build dauciform roots, were treated as if they could not build such root structures.

Species	Dauciform roots present	Dauciform roots absent	Reference
Carex canescens		1	Güsewell, unpublished
Carex davalliana		1	Shane et al. 2005
Carex echinata		1	Güsewell, unpublished
Carex elata		1	Shane et al. 2005
Carex flacca	1		Shane et al. 2005
Carex flava	1		Shane et al. 2005
Carex hostiana	1		Shane et al. 2005
Carex limosa		1	Shane et al. 2005
Carex nigra		1	Shane et al. 2005
Carex pallescens		1	Not known
Carex panicea	1		Shane et al. 2005
Carex pauciflora		1	Not known
Carex rostrata		1	Shane et al. 2005
Eleocharis sp.		1	Shane et al. 2005 (*E. acuta* and *E. palustris*)
Eriophorum angustifolium		1	Shane et al. 2005
Eriophorum vaginatum		1	As *E. angustifolium* and *E latifolium* do not build dauciform roots (Shane et al. 2005), E. vaginatum was assumed also not to do so.

Results

Influence of Fe, P and water supply on *Carex panicea* and *Schoenus nigricans*

Both dauciform root formation and biomass of all fractions were significantly influenced by phosphorus, however, not by iron or water level (Table 6 and Table 7). The number of dauciform roots per total biomass significantly decreased and the total biomass significantly increased with increasing phosphorus supply (Figure 1a and c). The total number of dauciform roots only differed between the highest and the two lower P supplies (Figure 1b). No effect of water table (Figure 1d-f), iron level and interactions with these two factors could be found (Table 6 and Table 7). *Carex panicea* and *Schoenus nigricans* significantly differed in all biomasses except the rhizome biomass. The difference in dauciform root formation between species was more pronounced when all phosphorus levels were included in the analysis; this was because the difference in cluster root number was greatest at lowest phosphorus supply and because *S. nigricans* built less cluster roots at high and more at low P supply compared to *C. panicea* (Table 4). Also the site of collection of *C. panicea* significantly influenced dauciform root formation and most biomasses; the two populations collected at Greifensee were similar in all responses and differed from the one collected at Neuchatel (data not shown).

Table 4: Means and standard error of dauciform root measures in dependence of species and phosphorus levels. P1: low phosphorus supply, P2: medium P supply, P3: high P supply.

		P1	**P2**	**P3**
No. of dauc roots	*Carex panicea*	184.59 ± 16.81	175.61 ± 12.03	14.62 ± 2.45
	Schoenus nigricans	71.73 ± 7.36	57.90 ± 5.91	34.00 ± 6.09
No. of dauc roots/	*Carex panicea*	201.70 ± 16.27	80.31 ± 5.05	4.90 ± 0.78
plant biomass (g)	*Schoenus nigricans*	56.27 ± 6.01	33.04 ± 2.90	17.00 ± 2.44

Root branching differed significantly among the three phosphorus levels (p<0.001) and between species (p<0.001); however, not among iron levels (p=0.395) and sites (P=0.518). Also water level did not have a significant influence on root branching (p=0.310). High P supply seemed to favour the formation of a herringbone root system; and *C. panicea* more often showed a herringbone root system compared to *S. nigricans* at all phosphorus levels (Table 5). All plants had either a two-order (i.e. herringbone) or a three-order root system. No plant showed a highly branched root system with more than three orders.

Table 5: Percentage of plants with herringbone or branched root system of phosphorus levels (P1-P3) and species.

	C. panicea			*S. nigricans*		
Root system	**P1**	**P2**	**P3**	**P1**	**P2**	**P3**
herringbone	0	18.6	65	0	0	6.7
branched	100	81.4	35	100	100	93.3

Table 6: Results of a two-way ANOVA of the effects of medium and high iron supply (Fe), medium and high phosphorus supply (P), water level, species and site (nested in species) on dauciform root formation and biomasses of *Carex panicea* and *Schoenus nigricans*. Three- and four-way interactions were not significant, thus, the simpler model up to two interactions was fitted. Levels of significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05.

Dependent variable	df	No. of dauc. roots	No. of dauc roots / rootbiomass	No. of dauc. roots/ plant biomass	shoot living	Shoot dead	roots	rhizome	total biomass	shoot:root
Fe	1	3.469 ns	1.772 ns	2.630 ns	0.246 ns	0.311 ns	0.593 ns	0.008 ns	0.367 ns	0.122 ns
P	1	277.349 ***	377.574 ***	387.260 ***	28.136 ***	0.203 ns	37.683 ***	15.167 ***	35.579 ***	1.090 ns
Water level	1	0.565 ns	1.570 ns	1.006 ns	0.643 ns	1.760 ns	0.021 ns	0.621 ns	0.040 ns	3.227 ns
species	1	6.471 *	0.541 ns	2.047 ns	57.811***	55.443 ***	73.359 ***	0.006 ns	40.238 ***	8.401 **
Fe*P	1	0.369 ns	0.092 ns	0.237 ns	0.079 ns	3.747 ns	0.077 ns	0.589 ns	0.164 ns	1.035 P
Fe*water level	1	1.458 ns	0.012 ns	0.348 ns	1.353 ns	0.523 ns	0.867 ns	0.020 ns	0.957 ns	0.135 ns
Fe*species	1	0.334 ns	0.000 ns	0.058 ns	2.085 ns	0.123 ns	1.567 ns	0.758 ns	1.599 ns	0.101 ns
P*water level	1	1.002 ns	2.521 ns	2.033 ns	2.701 ns	0.027 ns	2.601 ns	0.588 ns	2.055 ns	0.003 ns
P*species	1	63.920 ***	85.628 ***	79.459 ***	2.201 ns	0.094 ns	21.097 ***	2.579 ns	9.858 **	9.466 **
Water level*species	1	0.280 ns	0.146 ns	0.010 ns	1.109 ns	0.102 ns	2.004 ns	0.021 ns	1.099 ns	0.012 ns
Site[species]	2	10.913 ***	7.311 **	9.092 ***	0.0204 ns	2.969 ns	15.014 ***	21.611 ***	11.074 ***	30.826 ***
Fe*site[species]	2	2.816 ns	1.171 ns	1.938 ns	0.182 ns	1.627 ns	1.596 ns	1.372 ns	1.496 ns	1.566 ns
P*site[species]	2	1.128 ns	1.210 ns	1.908 ns	3.882 *	0.374 ns	6.545 **	3.846 *	5.653 **	0.147 ns
Water level*site[species]	2	2.606 ns	4.078 *	3.287 *	0.177 ns	5.047 **	0.387 ns	1.352 ns	0.304 ns	1.066 ns

Table 7: Results of a two-way ANOVA of the effects of three iron levels, three phosphorus levels, species and site (nested in species) on dauciform root formation and biomasses of *C. panicea* and *S. nigricans*. The three-way interaction was not significant, thus, the simpler model up to two interactions was fitted. Levels of significance: ***: p<0.001, **: p<0.01, *: p<0.05, ns: p>0.05.

| Dependent variable	df	No. of dauc. roots	No. of dauc. roots/ rootbiomass	No. of dauc. roots/ plant biomass	shoot living	Shoot dead	roots	rhizome	total biomass	shoot:root
Fe	2	0.938 ns	0.227 ns	0.117 ns	1.857 ns	1.324 ns	2.820 ns	0.254 ns	1.590 ns	2.235 ns
P	2	112.017 ***	230.553 ***	291.186 ***	143.689 ***	1.734 ns	96.221 ***	82.647 ***	131.907 ***	25.693 ***
species	1	23.708 ***	26.328 ***	5.979 *	20.148 ***	56.804 ***	29.926 ***	1.655 ns	12.880 ***	16.345 ***
Fe*P	4	1.511 ns	0.527 ns	0.707 ns	0.481 ns	2.864 *	0.412 ns	0.171 ns	0.430 ns	0.528 ns
Fe*species	2	1.068 ns	0.171 ns	1.713 ns	1.438 ns	0.924 ns	2.960 ns	1.903 ns	1.915 ns	0.242 ns
P*species	2	12.033 ***	30.607 ***	26.955 ***	13.416 ***	0.934 ns	37.857 ***	15.480 ***	25.639 ***	7.935 ***
site[species]	2	11.224 ***	5.987 **	12.805 ***	1.512 ns	16.488 ***	9.076 ***	18.977 ***	7.459 ***	39.702 ***
Fe*site[species]	4	0.716 ns	0.208 ns	0.339 ns	0.391 ns	1.031 ns	1.468 ns	0.942 ns	1.090 ns	3.509 **
P*site[species]	4	1.855 ns	2.490 *	3.429 *	0.811 ns	0.678 ns	1.181 ns	1.952 ns	1.188 ns	1.895 ns
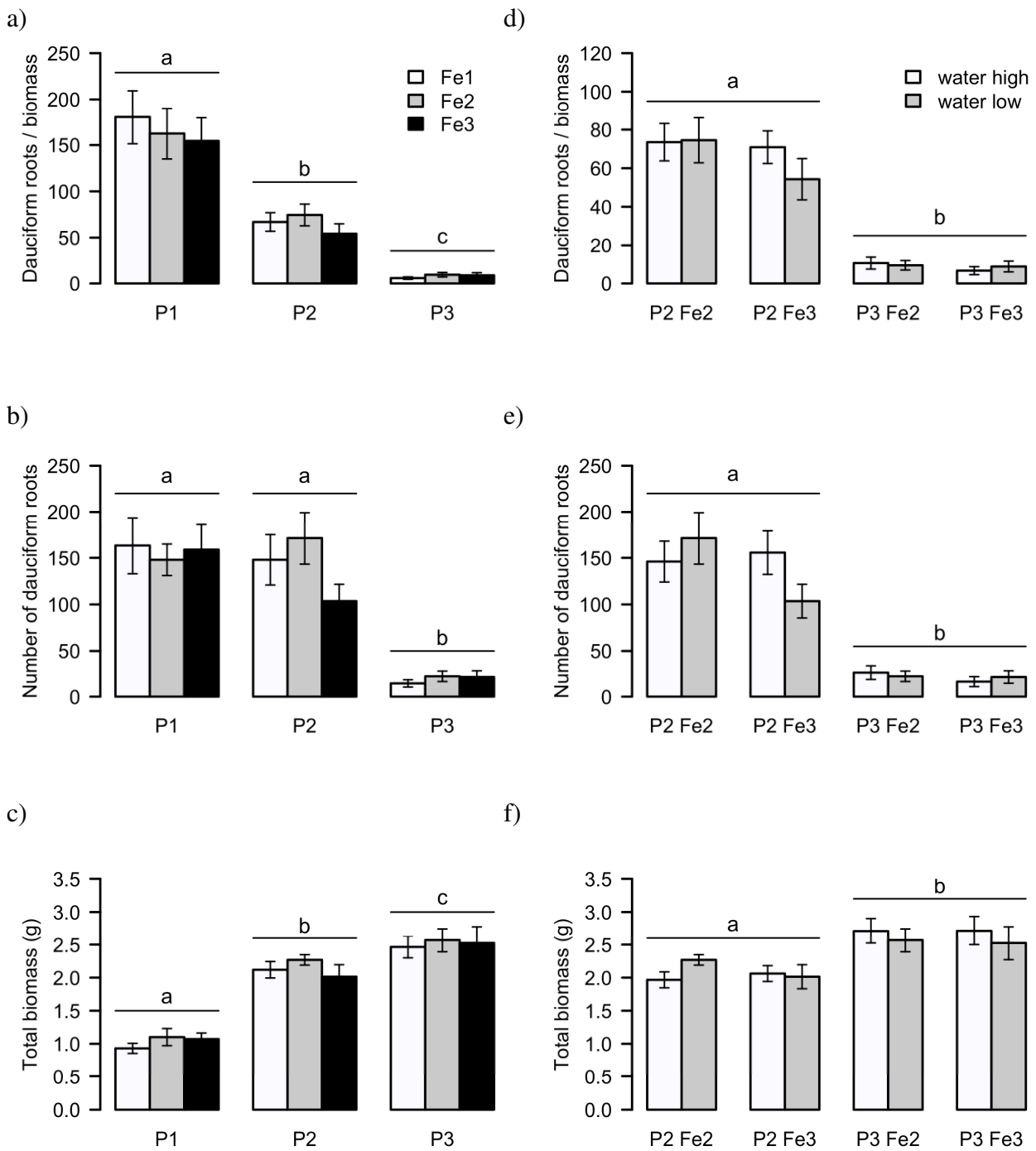
Figure 1: Influence of iron and phosphorus (a-c) and water table (d-f) on dauciform root formation and total biomass. P/Fe 1: low supply, P/Fe 2: medium supply, P/Fe 3: high supply. Different letters within one graph indicate significant differences (Tukey's HSD test, $p<0.05$). ANOVA results in Table 6 and Table 7.

Ecological niche of Cyperaceae species forming dauciform roots

Species forming dauciform roots significantly differed in iron content from species not building such root structures, however, not in phosphorus or nitrogen content or N/P-ratio (Table 8).

Mires significantly differed in nutrient contents as well as in N/P ratio (Table 9).

Phosphorus availability of plots could be better explained by Landolt values and mean peat depth than iron and nitrogen contents (Table 10). P availability was highly positively influenced by wet-dry cycles, and negatively influenced by pH and light availability. Iron availability was significantly higher at higher moisture. Fe and P availability were not correlated with each other (R^2: 0.018, p=0.120).

Only few plots contained species, which were able to build dauciform roots (14 out of 80 plots).

Table 8: Mean Fe, P and N content and N/P ratio in plant tissue of *Cyperaceae* species recorded in mires, and means of species forming /not forming dauiform roots. n is the number of species values included in the calculation of mean Fe content (n (Fe)) and phosphorus and nitrogen content (n (P,N)). Species included in the category "Species with dauci-form roots" are *C. flacca*, *C. flava*, *C. hostiana* and *C. panicea*. In the category "species without dauciform roots" all other species except *C. pallescens* and *T. alpinum* (as only one nutrient value of these species was obtainable) were included. For a comparison of the two categories a Wilcoxon test was conducted for Fe, P and N and N/P ratio.

	n (Fe)	n (P,N)	Fe (µg/g)	P (mg/g)	N (mg/g)	N/P
C. canescens	11	9	84.7 ± 14.2	1.9 ± 0.18	18.3 ± 0.7	10.3 ± 0.8
C. davalliana	4	0	68.1 ± 5.8	-	-	-
C. echinata	14	4	93.1 ± 14.2	0.9 ± 0.2	14.6 ± 0.6	18.5 ± 3.4
C. elata	7	7	52.9 ± 11.7	0.8 ± 0.05	13.7 ± 0.9	18.6 ± 1.9
C. flacca	3	1	243.4 ± 84.8	1.1 ± 0	14.8 ± 0	14.1 ± 0
C. flava	3	1	120.0 ± 15.4	0.7 ± 0	11.2 ± 0	15.3 ± 0
C. hostiana	6	2	189.0 ± 36.3	0.7 ± 0.06	13.5 ± 0.1	19 ± 1.5
C. limosa	7	6	65.1 ± 10.9	0.9 ± 0.1	16.9 ± 1.1	19.4 ± 3.1
C. nigra	28	25	67.4 ± 6.5	1.1 ± 0.1	18.3 ± 0.7	18.0 ± 1.2
C. pallescens	0	1	-	0.8 ± 0	14.1 ± 0	17.6 ± 0
C. panicea	10	7	88.0 ± 23.7	0.8 ± 0.1	13.5 ± 1	16.9 ± 1.3
C. rostrata	39	38	58.4 ± 6.4	1.1 ± 0.07	15.9 ± 0.4	15.6 ± 0.9
E. angustifolium	25	24	62.2 ± 7.2	1.0 ± 0.09	14.1 ± 0.6	15.9 ± 0.8
E. vaginatum	44	44	46.0 ± 4.5	1.2 ± 0.1	15.6 ± 0.3	14.0 ± 0.6
Eleocharis sp.	29	29	37.9 ± 2.8	0.7 ± 0.02	14.6 ± 0.4	21.0 ± 0.9
R. alba	2	1	148.2 ± 42.3	1.0 ± 0	19.7 ± 0	19.9 ± 0
S. sylvaticus	4	4	127.9 ± 55.4	1.0 ± 0.1	13.4 ± 0.8	15.3 ± 3.4
T. alpinum	1	0	3.79 ± 0	-	-	-
Species with dauciform roots	4	4	160.1 ± 34.8 a	0.8 ± 0.08 a	13.2 ± 0.8 a	16.3 ± 1.1 a
Species without dauciform roots	12	11	76.0 ± 9.5 b	1.1 ± 0.09 a	15.9 ± 0.6 a	17.0 ± 0.9 a

Table 9: Mean Fe, P and N availability and N/P of mires. Means are calculated from plot nutrient availabilities. n=10.

Mire	Fe (µg/g)	P (mg/g)	N (mg/g)	N/P
Amden	87.37 ± 4.03	0.74 ± 0.03	1.61 ± 0.07	22.37 ± 1.11
Breuleux	106.55 ± 11.95	1.37 ± 0.09	1.88 ± 0.04	13.38 ± 0.53
Gruère	85.79 ± 4.53	0.72 ± 0.05	1.75 ± 0.09	20.64 ± 0.91
Mettilimoos	81.12 ± 5.66	0.99 ± 0.06	1.83 ± 0.06	14.21 ± 0.58
Oberiberg	110.99 ± 20.63	0.98 ± 0.05	2.44 ± 0.04	16.14 ± 1.04
Salwidili	102.43 ± 6.06	0.82 ± 0.05	1.49 ± 0.06	17.92 ± 0.63
Schwendisee	91.98 ± 8.58	1.03 ± 0.06	2.3 ± 0.13	15.38 ± 1.46
Sud des Veaux	102.61 ± 9.28	1.32 ± 0.06	1.6 ± 0.02	13.71 ± 0.58

Iron and phosphorus did neither have an influence on the number of species forming dauciform roots in a plot, nor on the cover of species forming dauciform roots in a plot (Figure 2).

The presence of cluster forming species was neither dependent on iron, nor on phosphorus availability (Table 11). However, their occurrence was positively associated with increasing pH, D Landolt value, indicating soil oxygen supply, and N Landolt value. On the contrary, humus Landolt value, indicating the amount of mould in the soil, and mean peat depth appeared to have a negative effect on the presence of cluster forming species. Temperature and moisture influenced the occurrence of dauciform root forming species positively and negatively, respectively, however, only weakly. The factors associated with the presence of cluster forming species were highly correlated with each other.

Species with the ability of forming dauciform roots occurred in all plant communities; they were not restricted to one vegetation type (Figure 3).

Table 10: t-values of stepwise regressions to identify variables best explaining nutrient contents of plots. Variables included in the model are Landolt indicator values for each plot (T: temperature, K: continentality, L: light, F: soil moisture, W: wet-dry cycle, R: pH, N: soil nutrient availability, H: amount of mould in the soil, D: soil oxygen supply) and mean peat depth of each plot. Mean pH was excluded from the analysis, as it was highly positively correlated with R Landolt value. Levels of significance: ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, ns: $p>0.05$.

	Fe	**P**	**N**	**N/P**
Intercept	4.102 ***	-1.741 ns	12.887 ***	4.806 ***
T	-1.916 ns	3.055 **	-3.262 *	-4.104 ***
K	-2.446 *	2.228 *		-2.978 **
L	-2.628 *	-3.690 ***		2.767 **
F	2.791 **	2.560 *		-1.779 ns
W		4.825 ***		-4.657 ***
N			2.791 *	
R	2.513 *	-3.354 **		3.487 ***
D	1.706 ns	1.731 ns		-1.414 ns
H		-1.931 ns		1.623 ns
mean peat depth			3.057 *	
Model R^2	0.107	0.368	0.133	0.353

Table 11: Results of the analysis of deviance of a logistic model (binomial) of the effects of nutrient availability, indicator values, mean pH and peat depth on the presence or absence of species forming dauciform roots. Every site condition was analyzed separately. Abbreviations for Landolt indicator values: T: temperature, K: continentality, L: light, F: soil moisture, W: wet-dry cycle, R: pH, N: soil nutrient availability, H: amount of mould in the soil, D: soil oxygen supply.

| site conditions	Df	Deviance	Resid. Df	Resid. Dev	P(>|Chi|)	relationship
NULL			79	74.196		
Fe availability	1	2.663	78	71.533	0.103	
P availability	1	0.023	78	74.173	0.879	
N availability	1	0.747	78	73.449	0.388	
N/P ratio	1	0.076	78	74.121	0.783	
T	1	9.273	78	64.923	**0.002**	positive
K	1	1.088	78	73.108	0.297	
L	1	0.063	78	74.133	0.802	
F	1	8.996	78	65.200	**0.003**	negative
W	1	0.290	78	73.906	0.590	
R	1	35.555	78	38.642	**<0.001**	positive
N	1	24.189	78	50.007	**<0.001**	positive
H	1	51.013	78	23.484	**<0.001**	negative
D	1	10.013	78	63.197	**<0.001**	positive
Mean pH	1	56.684	78	17.513	**<0.001**	positive
Mean peat depth	1	30.336	78	43.860	**<0.001**	negative
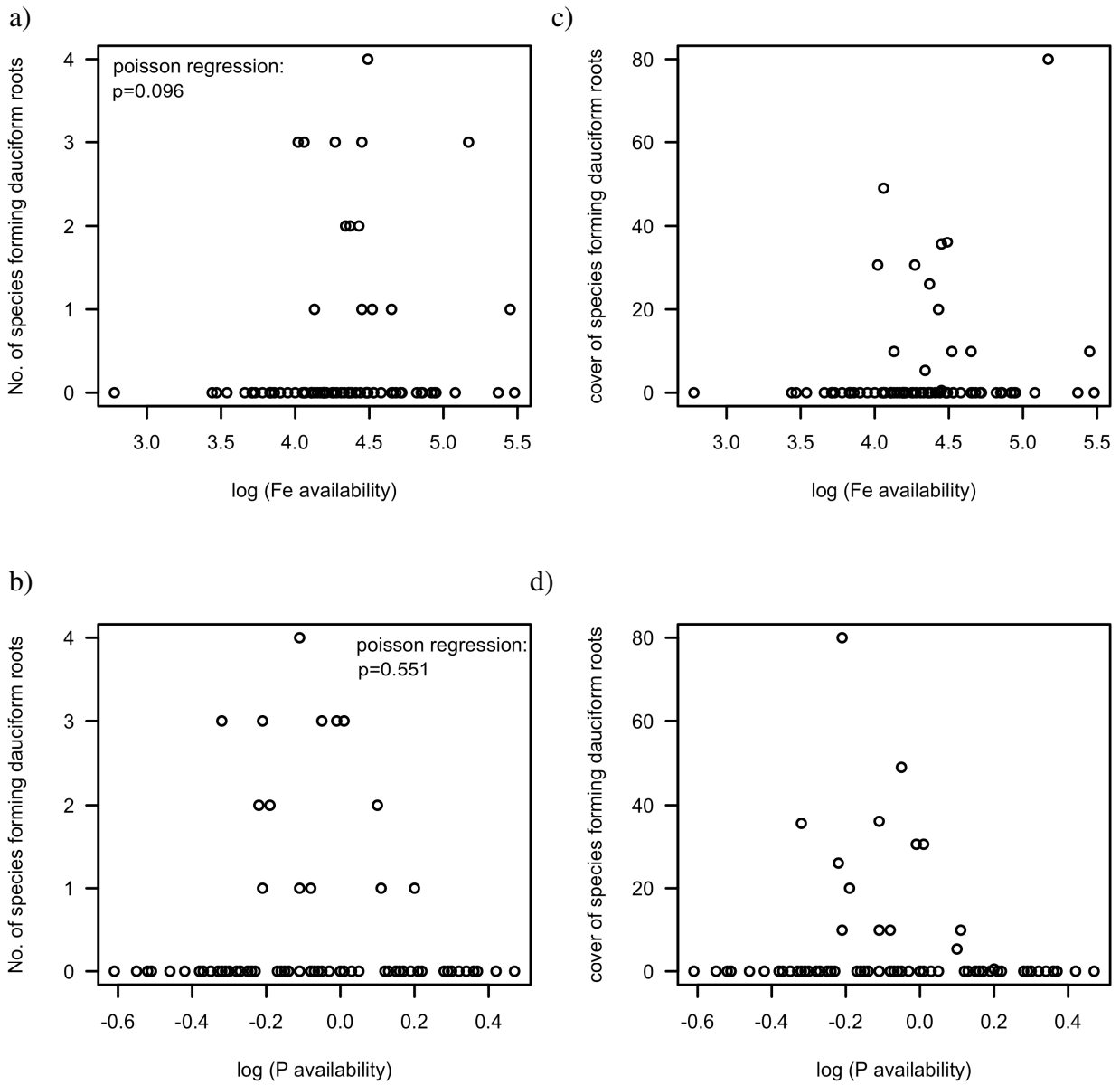
Figure 2: Relation of Fe or P availability and number of Cyperaceae species forming dauciform roots (a, b) or the total cover of species forming dauciform roots (c, d) in each plot. Number of plots: 80.
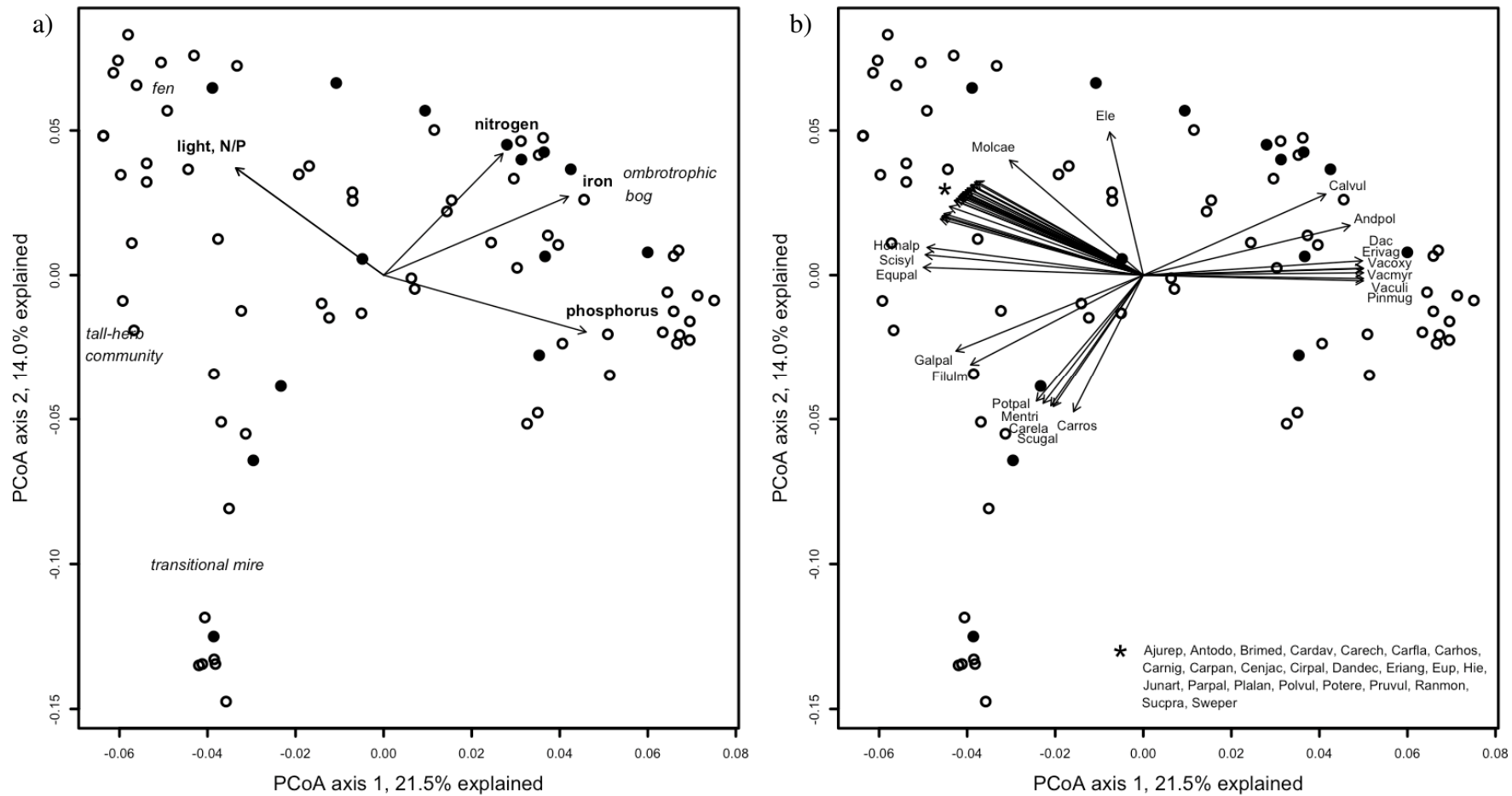
Figure 3: Principal Coordinates Analysis (PCoA) ordination diagram of vegetation relevés (n=80) with the environmental variables (a) and species (b), which correlate with the first two ordination axes ($p<0.05$). Closed circles: plots with species forming dauciform roots; open circles: plots without species forming dauciform roots. In italic are the plant communities defined by the occurring plant species (b). Species in figure b: *Ajuga reptans, Andromeda polifolia, Anthoxantum odoratum, Brizza media, Calluna vulgaris, Carex davalliana, Carex echinata, Carex elata, Carex flacca, Carex hostiana, Carex nigra, Carex panicea, Carex rostrata, Centaurea jacea, Cirsium palustre, Dactylorhizza sp., Danthonia decumbens, Eleocharis sp., Equisetum palustre, Eriophorum angustifolium, Eriophorum vaganiatum, Euphrasia.sp., Filipendula ulmaria, Galium palustre, Hieracium sp., Homogyne alpina, Juncus articulatus, Menyanthes trifoliate, Molinia.caerulea, Parnassia palustris, Pinus mugo, Plantago lanceolata, Polygala vulgaris, Potentilla erecta, Potentilla palustris, Prunella.vulgaris, Ranunculus montanum, Scirpus silvaticus, Scutellaria galericulata, Succisa pratensis, Swertia perennis, Vaccinium myrtillus, Vaccinium oxycoccus, Vaccinium uliginosum.*

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Discussion

Influence of Fe, P and water supply on dauciform root formation

Only phosphorus level significantly influenced dauciform root formation of both *Carex panicea* and *Schoenus nigricans*. The importance of phosphorus for the formation of dauciform roots is widely acknowledged. Shane et al. (2005) also found a decrease in dauciform roots with increasing phosphorus supply up to the suppression at highest P level in *Schoenus unispiculatus*. The same pattern was found for *Carex flacca* and *Caustis blakei* (Bakker et al. 2005, Playsted et al. 2006). In contrast to my results for *Schoenus nigricans*, Bakker et al. (2005) found dauciform root production of this species to be independent of phosphorus supply. These opposing results reflect the fact that internal rather than external P status determines dauciform root production (Bakker et al. 2005, Shane et al. 2005, Shane et al. 2006). Apparently, both species investigated in the present study experienced internal phosphorus deficiency. However, *C. panicea* formed more dauciform roots at low and less at high phosphorus concentrations compared to *S. nigricans*. Thus, *C. panicea* generally seems to be more plastic in terms of dauciform root formation than *S. nigricans*.

Surprisingly, iron supply did neither affect dauciform root production nor biomasses of either species. As iron supply has been found to significantly influence growth of *Carex* and other species (Snowden and Wheeler 1993, Batty and Younger 2003, Nenova 2009) this result may indicate that plants did not experience Fe deficiency or toxicity at any supply level. Possibly, iron was supplied in the wrong form to induce a response in dauciform root formation. Arahou and Diem (1997) detected that in *Casuarina glauca* (Casuarinaceae) iron deficiency played a greater role in cluster root induction than phosphorus, however, only when an iron chelator was added leading to effective iron-stressed conditions. But also the effective form of iron supply may have influenced the result. Nitrogen supply, for instance, does not seem to affect cluster root formation, whereas N source does (Racette et al. 1990, Arahou and Diem 1997). Likewise, cluster production can depend on phosphorus mineral source (Shane et al. 2008). Another possibility is that dauciform root formation does not depend on iron supply in the investigated sedge species. Iron deficiency did not promote cluster root formation in *Banksia ericifolia* (Proteaceae) (Handreck 1991), *Lupinus albus* (Fabaceae) (Watt and Evans 1999), *Myrica cerifera* L. (Myricaceae) (Louis et al. 1990) and *Alnus incana* (Betulaceae) (Watt and Evans 1999). Furthermore, only one species (*Lupinus consentinii*) was found to respond to both phosphorus and iron deficiency with enhanced cluster root production (Watt and Evans 1999). Thus, it is conceivable that also *C. panicea* and *S. nigricans* do not respond to different iron supplies.

Water level did not influence dauciform root formation and biomass of *Carex panicea* and *Schoenus nigricans* as well. Possibly, the two water levels were not distinct enough to affect plant growth. However, plants grown at high water table did not root as deeply as plants grown at low level (personal observation), indicating that water levels did influence growth indeed. Davies (1970), cited in Davies et al. (1973), found that the number of dauciform roots in *Carex panicea* and *C. flacca* was very low in extremely dry habitats. He explained this result by the relatively unbranched root system developed by *Carex* species under drought conditions. In the present study, neither of the two water treatments simulated dry conditions. Thus, the findings of the present study and of Davies (1970) do not contrast each other. The hypothesis that cluster root formation is only affected by water availability when very dry and wet conditions are compared, is supported by the study of Lamont (1976). This author examined the effect of water availability on cluster root formation of *Hakea* species. He showed that the number of cluster roots per total root weight increased by the threefold under waterlogged conditions. However, the increase was only significant for very low to medium water availability (below field capacity to 1-2 times field capacity); there was no difference between medium to high water availability (1-2 to 4-6 times field capacity). Also Poot and Lambers (2003) found cluster root formation of *Hakea* species to be dependent on water level. These authors also found an effect of water logging on shoot dry biomass, which contrasts to the findings of the present study. This could be explained by the optimal growth of both species under

wet conditions (Landolt in press, in press). It is probable that both treatment levels lay in the optimal conditions range for both species, as they did not experience dry conditions at any time.

Ecological niche of Cyperaceae species forming dauciform roots

Species forming dauciform roots were found in all plant communities investigates. Thus, these Cyperaceae species do not seem to fill a special ecological niche, but can be present in any vegetation type.

With this data set, no connection between nutrient availability and presence of cluster-bearing Cyperaceae could be found. Consistent with the pot experiment, the occurrence of species producing dauciform roots seems to be independent of iron availability. Probably, there is no scarcity of plant available iron in the investigated mires. Iron concentration in the soil increases with decreasing pH and under anaerobic conditions soluble iron may even accumulate to toxic conditions (Mengel and Kosegarten 2005). Thus, a mechanism for iron acquisition may not be necessary in wetland sedges. Species forming dauciform roots had a higher mean iron content in plant tissues. From this result, however, the conclusion that these species are better supplied with iron can not be derived, as iron can be accumulated in leaves in physiologically less active forms in some species which are not well supplied with iron (Zohlen and Tyler 1997, 2000).

Surprisingly, the occurrence of cluster-bearing Cyperaceae species was not found to depend on phosphorus availability. In contrast to this result, Lambers et al. (2006) found a nutrient niche for non-mycorrhizal, cluster-bearing Proteaceae; they mainly occurred at phosphorus-impoverished sites, whereas mycorrhizal Myrtaceae without cluster roots dominated sites with higher phosphorus availability in south-western Western Australia. It must be pointed out that Proteaceae and Myrtaceae did not replace one another but also co-occurred (Lambers et al. 2006). The question is, why species forming a root structure which is believed to enhance phosphorus availability (e.g. Playsted et al. 2006, Shane et al. 2006) did not mainly occur at sites with lower P availability in the present study. There are three possible explanations. First, phosphorus availability might have been to high to favor species forming dauciform roots and to exclude species not building such root structures. Second, non-cluster-bearing sedge species might exhibit other morphological adaptions to phosphorus poor conditions and thus are also able to colonize such sites (Davies et al. 1973). For instance, some *Carex* species as *C. diandra* (Davies et al. 1973) exhibit a highly branched root system which enhances phosphate uptake (Fitter et al. 2002). Also the formation of a deep root system (as *Carex arenaria* does) exploiting other soil layers than are typical for cluster-bearing species could be a possibility to cope with low nutrient availability (Davies et al. 1973). Third, nutrient contents of plants used to estimate nutrient availability of plots might not reflect real soil nutrient availability. Despite that plant nutrient content is, especially in agricultural science, widely used to estimate soil nutrient availability (e.g. Van Duren and Pegtel 2000, Rozbrojova and Hajek 2008), there is considerable debate on whether this measure really reflects nutrient availability of a site (Wassen et al. 1995). Nutrient contents of plant tissue might not reflect soil nutrient availability due to luxury consumption or nutrient allocation to storage organs (Van Duren and Pegtel 2000). There are contrasting results concerning this question. For instance, Nenova and Stoyanov (1999) found that iron concentration in leaves of young maize plants did not reflect the amount of iron supplied. However, high and low iron supplies resulted in high and low iron concentrations respectively. In opposition to this result, Snowden and Wheeler (1995) who investigated shoot iron concentrations of four wetland species found a significant increase of Fe concentration with increasing Fe supply. In the present study, the speculation that nutrient contents used to estimate plot nutrient availability do not reflect soil nutrient availability is supported by the fact that the nutrient availabilities are highest in ombrotrophic bogs (Figure 3); a habitat which is extremely nutrient poor, much more compared to other mires as a result of eluviation (Baltisberger 2003).

Noteworthy is that the occurrence of species producing dauciform roots is associated with pH (positively), Landolt nutrient (positively), oxygen supply (positively) and humus (negatively) value and weekly with Landolt moisture value (negatively). These factors are highly correlated with each

other; mires with higher pH exhibit higher nutrient availability and less humus and often are less wet and thus have a higher oxygen supply. Contrary to this result, the cluster-forming species *Carex panicea* has been found to produce dauciform roots in the field under acid peat in England (Davies et al. 1973); a situation, which can not be found in Switzerland as *C. panicea* only occurs in neutral to basic conditions in Switzerland (Sabine Güsewell, ETH Zurich, personal communication, Landolt, in press). In this data set, only few plots with cluster-forming Cyperaceae have been found, as mainly extremely acid mires have been sampled, not fulfilling the conditions mentioned above. Thus, only limited conclusions about the ecological niche of dauciform root producing species can be derived from this data set.

Conclusions

Dauciform root formation of Cyperaceae species seems not to depend on iron availability. Phosphorus seems to be of much higher importance than iron, not only in the formation of dauciform roots, but also in biomass production and root branching, as the pot experiment showed. Species forming dauciform roots occured in all vegetation types investigated and thus do not seem to fill a special ecological niche, as the cluster-bearing Proteaceae in south-western Western Australia do (Lambers et al. 2006). However, species producing dauciform roots occur at the upper pH range of the sites investigated in this study, indicating a major occurrence in mires with higher pH than sampled in the present study. To get a deeper insight into the ecological niche of cluster-bearing Cyperaceae, less acid mires should be examined.

Acknowledgements

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