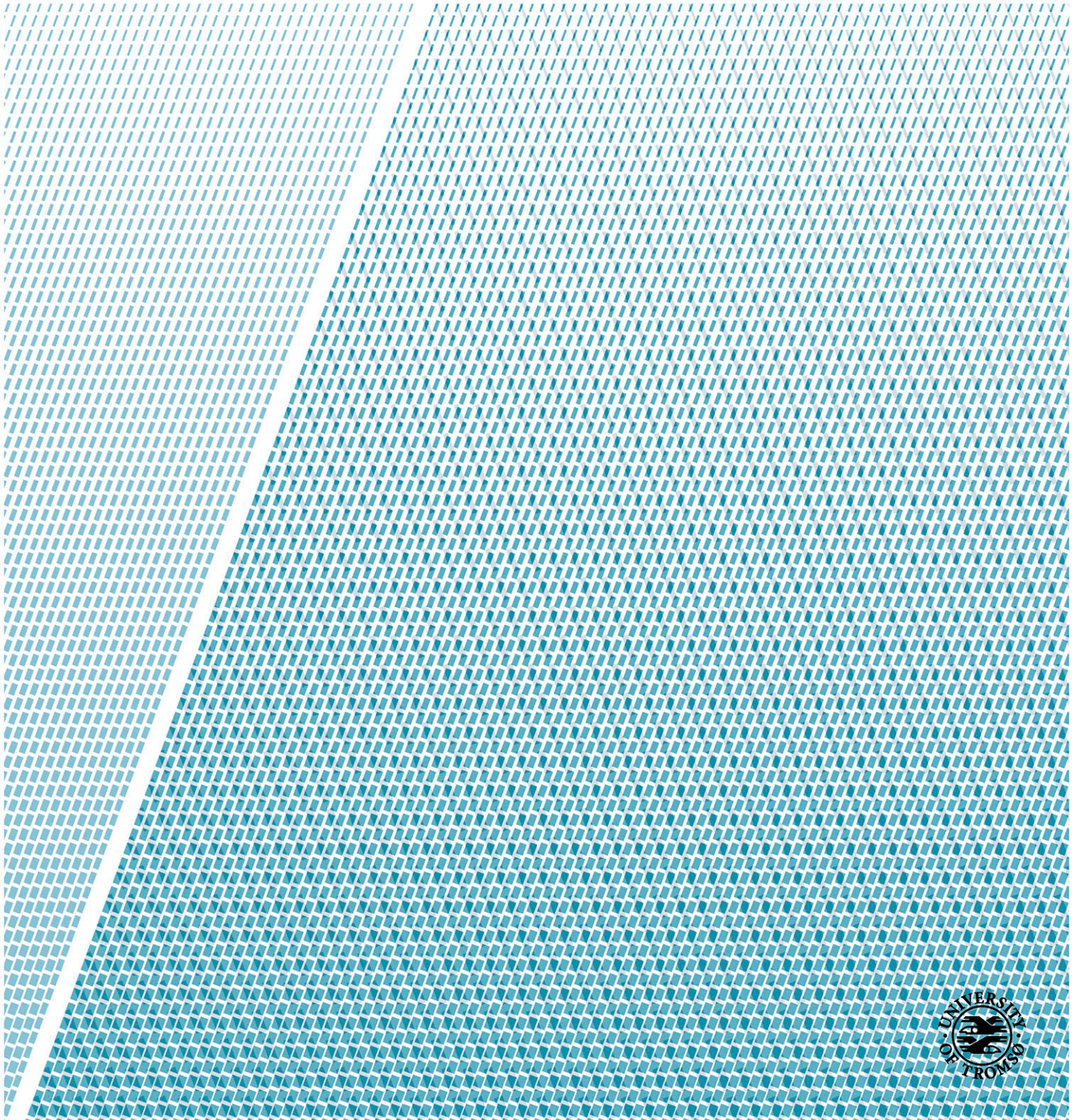


Initial Community Convergence on Plant Defense Syndromes Explains Community Responses to Herbivore Exlosures

Jacob Robinson

BIO-3950 Master Thesis in Biology, Northern Populations and Ecosystems

November 2017



Faculty of Biosciences, Fisheries and Economics

Department of Arctic and Marine Biology

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Supervisor:

Jutta Kapfer, NiBio – Norwegian Institute of Bioeconomy Research

Co-Supervisors:

Kari Anne Bråthen, UiT - The Arctic University of Norway



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Abstract

General trade-offs in species' energy use are highlighted here by mapping proposed defense syndromes, using trade-offs found between: constitutive phenolic compounds, silica and a high nutrient low defense region found between regional con-specifics.

Differences in species composition between two catchments can be explained by proposed syndrome trade-offs seeking energy use optimums in differing (a)biotic conditions.

Mapping compositional change resulting from herbivore exclosure experiments, as trade-offs in identified "defense" syndromes between con-specifics when a constraint is lifted, yields a dimension beginning to populate a less constrained fitness landscape.

However, at the same time other (a)biotic forces continue to push the system to a resource use optimum. In this system, changes conspire to move catchments in a similar direction.

It is proposed that defining and discovering trait suites occurring from (a)biotic trade-offs, are best modeled by finding trade-offs at other scales; and that modeling them through time produces a viable model of speciation/convergence occurring through a fitness landscape.

Keywords: coevolution, phenolics, silica, plant defense, plant-herbivore interaction, chemical ecology, defense syndromes.

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1 Introduction

Vertebrate herbivore exclosure experiments from 2006 to 2008 caused significant compositional change in tundra grasslands on riparian plains in the Varanger Peninsula, Norway (Ravolainen et al., 2011). Results were context dependent: with herbivore exclosure increasing forbs and decreasing silica rich grasses in one catchment, and increasing forbs and deciduous shrubs in another. It is thought that such herbivore induced changes result from species being released from grazing pressure, wherein species resistant to grazing become less competitive than species unresistant to grazing; and species tolerant to grazing becoming less competitive than species intolerant of grazing. This change in competitiveness assumes costs, in that tolerance or resistance to herbivory limits resources or opportunities for greater growth and reproduction, an assumption fundamental to a number of plant defense theories (Bazzaz et al., 1987; Bryant et al., 1983; Coley et al., 1985; Feeny, 1976; Gulmon & Mooney, 1986; Herms & Mattson, 1992; Rhoades, 1979; Stamp, 2003); and found in a majority of studies (Strauss et al., 2002). However, exactly which of the myriad plant defense theories best explains patterns seen in nature is still contested, and confusion has arisen over exactly what those theories should predict (Endara & Coley, 2011; Stamp, 2003).

The concept of plant defense syndromes has therefore been proposed as a useful model to understand how biotic and abiotic forces influence defensive strategies in plants (Agrawal & Fishbein, 2006). It suggests that plant defenses are best understood, not as single traits, but as broad arsenals organized into suites of co-varying traits. These suites converge into what are termed “syndromes” It postulates that “...within a regional community, plant species can converge on a few defense syndromes, yet the divergent strategies (across the syndromes) also can promote the coexistence of species.” In a study of 24 *Asclepias* species Agrawal and Fishbein (2006) found evidence of 3 species clusters representing 2 distinct defense syndromes—high nutrition/high defense, and low nutrition/low defense. A third syndrome, tolerance/escape, is predicted to exist, but does not occur with in the *Asclepias* species studied. Figure 1 was presented as an outline to their findings, in which species cluster ‘B’ was characterized as inhabiting the “Low nutritional quality” defense syndrome while species clusters ‘A’ and ‘C’ inhabited the “Nutrition and

defense” syndrome. It is important to note that species clusters ‘A’ and ‘C’ both inhabit the same syndrome despite having different defensive suites—‘A’ using physical defenses (trichomes and latex), and ‘B’ using chemical defenses (cardenolides). This indicates that an event in the history of the *Asclepias* genus produced trade-offs between energy allocation of trait suites that ultimately resulted in speciation. However, other events pushed those species to converge on a similar overall strategy.

Energy is the penultimate limiting resource for any organism, and trade-offs in its allocation are therefore essential in modeling fitness landscapes. However, the plant defense syndrome model therefore cautions against the simplistic use of trade-offs in modeling plant defenses because many of the defensive traits that inevitably must trade-off against each other are difficult to observe, have escaped attention, or are not accounted for. However, because of fundamental resource constraints, trade-offs must be observable at some higher (or more inclusive) level of organization; and occur both between strategies (example: between species clusters ‘A’ and ‘B’) and between syndromes (example: between “Nutritional and defense” and “Low nutritional quality”).

This thesis finds that, among species, trade-offs occur between two defensive strategies inhabiting one syndrome region—between silica (chemo-mechanical) and constitutive phenolic (chemical) defenses inhabiting the “Nutrition and defense” syndrome region. This separation suggests phylogenetic divergence in strategy, but non-phylogenically related convergence on a particular syndrome region. It also finds trade-offs among species between constitutive phenolics/silica (SPCs/Si), and nitrogen/phosphorus (N/P) that identify the “Nutritional and defense” and “Tolerance/escape” regions of figure 1.

Furthermore, initial community composition in each catchment suggests community convergence on syndromes that maximize fitness against regional (a)biotic forces; and that this explains the different community responses to herbivore exclosure experiments—supporting the PDS model as a useful framework for asking deeper ecological questions.

2 Materials and Methods

This study utilizes ecological data and NIRS calibration data obtained from two previous studies summarized below. It adds NIRS calibrations for stable water-soluble phenolic compounds (SPCs) based on samples obtained for a previous NIRS calibration for Si (Smis et al., 2014). They are defined as “stable” because of the time elapsed between the

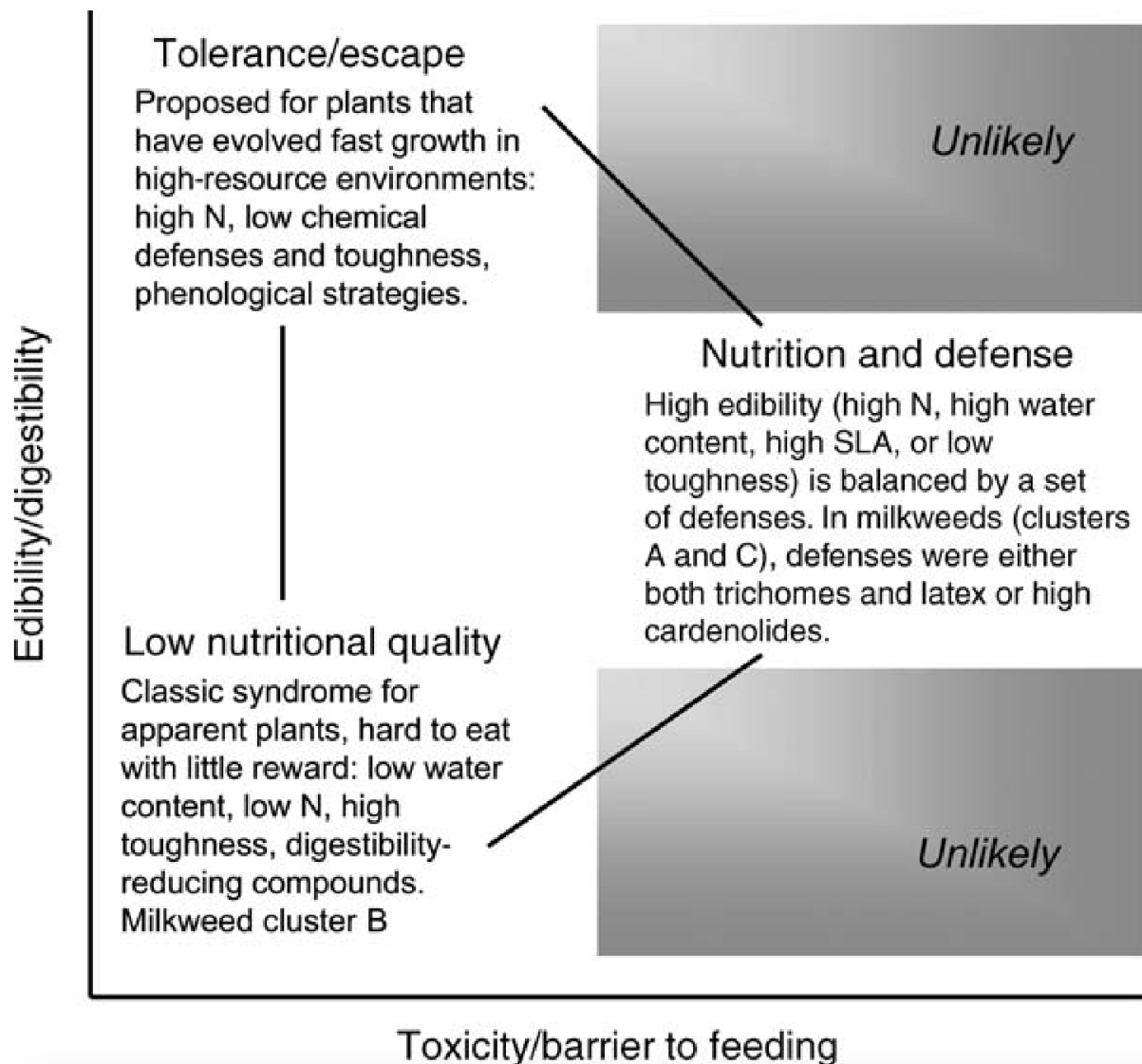


Figure 1: The plant defense syndrome triangle as presented by Agrawal & Fishbein (2006) characterizing 3 distinct plant defense syndromes. Evidence for both the “Nutrition and defense” and “Low nutritional quality” regions was found in a study of 24 *Asclepias* which grouped into 3 distinct species clusters (A, B, and C) based on an analysis of common plant defense suites. Note that species clusters ‘A’ and ‘C’ both inhabit the “Nutritional and defense” syndrome, but follow two different strategies; one trichome/latex based, the other cardenolide based.

NIRS scan and the calibration, and would likely fall the category of constitutive phenolic defense.

Ecological Data

Ecological data was taken from Ravolainen et al. (2011), which consists of data collected from 2006 to 2008 in the Vestre Jakobselv (VJ) and Komagdalen (KO) river catchments in northeastern Norway (70° - 71° N and 28° - 31° E) (Fig. 2). In each catchment sampling grids ($15\text{m} \times 15\text{m}$) were distributed (12 in KO and 13 in VJ) along a distance of 10-20km, with each grid bordered on one side by tall *Salix*, and meadow on the other three. Within each grid, 9 experimental plots ($0.5\text{m} \times 0.5\text{m}$) were established with 3 replicates of 3 randomly assigned treatments; (1) exclusion of all vertebrate herbivores using small mesh ($1\text{cm} \times 1\text{cm}$) cages, (2) exclusion of large vertebrate herbivores using large mesh ($3\text{cm} \times 3\text{cm}$) cages, and (3) unenclosed plots. All plots were centered around a *Salix* individual as part of another study design (Ravolainen et al., 2014). Plots were established in the first week of July, 2006 and the first measures of biomass taken approximately 3-4 weeks later. Further measurements were taken during the last week of July/first week of August in 2007 (data not shown) and 2008. Vegetation was measured using the point intercept frequency method (Bråthen & Hagberg, 2004), and converted to biomass using the calibration equations in table 8.

NIRS Phenolic Compounds Determination

Phenolics calibrations were prepared using plant samples obtained for previous plant Si calibrations (Smis et al., 2014). For SPCs analysis plant material was dried, milled and $20 \pm 5\text{mg}$ of material put into 2ml Eppendorf tubes and 1.6ml of methanol:water (80:20) solution added. The extract was put in a chilled ultrabath for 25min and then frozen at -20°C overnight. The solution was then centrifuged (10min at 13,000 rpm) to separate plant material and the supernatant solution transferred to a test tube. A 1.6ml volume of methanol:water (80:20) was again added to the plant material and mixed on a vortex mixer for 2h. The mixture was again centrifuged (10min at 13,000 rpm) and the supernatant transferred to the glass tube containing the first extraction. The process was repeated once more so that 3 extractions were combined. The extract was put in a speed vac system until dry, and 1ml of water added to dissolve the sample. The sample was then mixed in a vortex mixer for 5min and centrifuged, and the supernatant then

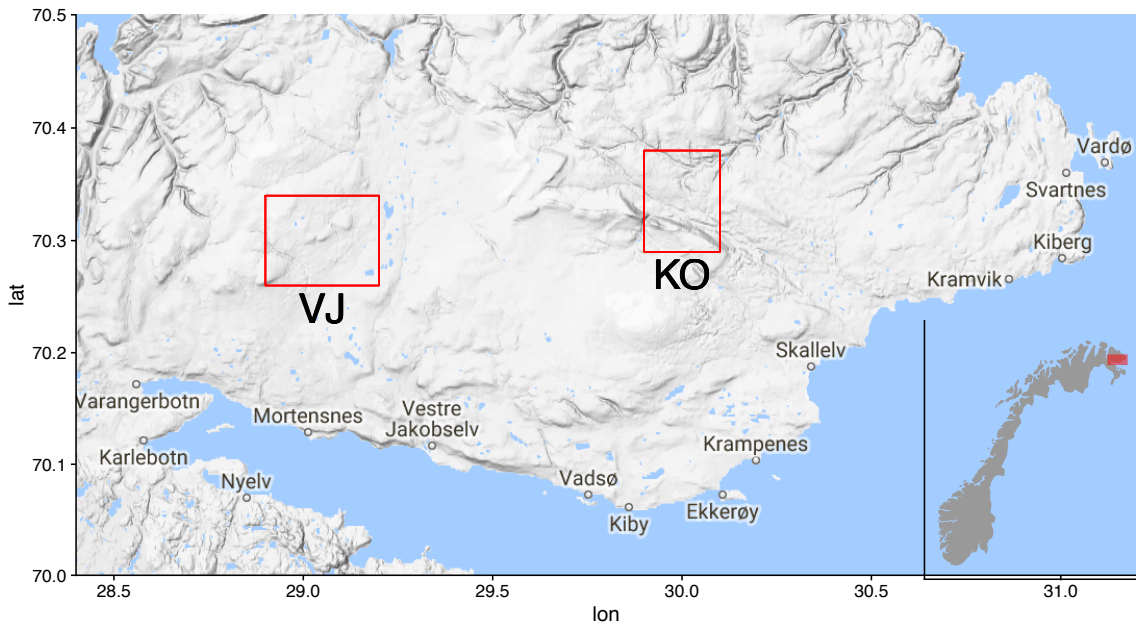


Figure 2: Map of Komag (KO), and Vestre Jakobselv (VJ) study sites in Varanghalvøya, Norway

pipetted into a new Eppendorf tube.

Total phenolics were measured according to Waterhouse (2002). Wherein 10 μ l of the above phenolic extract (or standard), 790 μ l of water and 50 μ l of Folin-Ciocalteu reagent were mixed in an Eppendorf tube and allowed to stand 1-8min (2 replicates for each sample). Sodium carbonate solution (150 μ l of 20% m/v) was then added and the solution allowed to stand 2h. 100 μ l of the mixture was then added to one well of a 96-well plate (3 replicates per Eppendorf tube), and the absorbance read at 765nm. Standards were created by first dissolving 1g of gallic acid with several drops of ethanol and then diluting with 500ml of purified water. Serial dilutions by half were then made from 2mg l⁻¹ to 0.125mg l⁻¹, as well as pure water.

The values thus obtained were used to calibrate previous NIRS readings from Smis et al. (2014) to find SPCs values. All values should be considered as gallic acid equivalents. NIRS readings were also used to determine N, P and Si values. The plant material obtained by Smis et al. (2014) was originally dried, milled, and pressed into tablets (16mm x >1mm) using 6 tons of pressure. Three scans of each sample were taken with a 16mm adapter and the mean recorded for that sample.

Additional, plant material was obtained in June 2016 from the KO, VJ and Ifjord

regions and dried on collection. Approximately 5 months after collection, the plant material was milled and scanned using a 4mm adapter used for powdered material. As no calibration was made directly from wet material to 4mm powder scans, additional work (TOMASSI project) was used to find a correction factor for 4mm scans to 16mm model calibrations. This consisted of scanning tableted plant material first with 16mm adapter, then powdering the tablets and re-scanning with 4mm adapter, and correcting 4mm readings to closely match 16mm readings (Fig. 6).

Statistics

NIRS Measures

Values obtained from NIRS were highly variable, even when 4mm scans were excluded. All negative values in the NIRS measurements were, therefore, adjusted to zero for SPCs, C, N, and P, while a cut-off value of 0.00024 given to Si (Smis et al., 2014). For calculations of community weighted means (CWM), species were assigned the median value from associated samples. The chemical values for species not scanned with NIRS but present in the ecological data were given the values of the most closely phylogenically related species that were analyzed, or an average of the genus/es most closely related.

Non-metric multi-dimensional scaling (NMDS) using Euclidian distance was used to plot species chemical values against each other. Linear mixed effects (LME) models using species within functional groups as a random factor were used to find statistically significant correlations between SPCs, Si, C, N, and P.

Community Measures

CWM were calculated according to the following equation:

$$\sum_{i=1}^S \frac{b_i}{(\sum_{i=1}^S b_i)} \cdot c_i$$

where S is the species b is biomass and c is the chemical measure (SPCs, C, N, P, or Si) (Roscher et al., 2012).

LME was used to model the response of variables: CWM SPCs, CWM Si, CWM N and CWM P, to treatment, year and catchment. Moreover, plant communities were separated into functional groupings: deciduous woody, *Salix*, evergreen woody, evergreen non-woody, hemiparasite, nitrogen fixer, forb, sedge, grass and cryptogram. Year was included in all

models as an interaction term to account for non-treatment related changes during the study period. Site was included as a random effect to account for differences between locations. LME model statistics were performed using the NLME package in R (Pinheiro et al., 2017; R Core Team, 2017).

NMDS scaling using the Bray-Curtis dissimilarity measure was used to plot the CWM chemical values against study sites. Adonis (permanova) analysis was used to determine significance of catchment, treatment and year groupings. Ordination and adonis statistics were performed with the *vegan* package in R (Oksanen et al., 2017).

3 Results

NIRS Measures

Clear differences in SPCs content were found between species and between functional groups (Fig. 3). SPCs, Si and N values were all significantly negatively correlated with each other (Table 1). Among species, the highest SPCs values were found among woody deciduous and *Salix* functional groups. Variability was also highest in these two groups. With some exceptions, the lowest SPCs values were found among species belonging to the sedge, grass, and cryptogram functional groups (Fig. 5).

Si content was high in graminoids and cryptograms, but surprisingly, the highest median values were in the hemiparasite and nitrogen fixing functional groups, however, sample sizes were small.

NMDS using Euclidean measure of all species SPCS, Si, N, and P values separated functional groups into 3 overlapping regions (Fig. 3). Si and SPCs regions are suggested to correspond to the “Nutrition and defense” region of figure 1. The N/P region is suggested to correspond to the “Tolerance/escape” region of figure 1. The region low in SPCs, Si, C and P is suggested to represent the “Low nutritional quality” region of figure 1.

Community Measures

Species inhabiting KO significantly differed from those in VJ in their initial CWM-SPCs, and N/P content. KO species (as a community) were higher in Si (though not significant, $p=0.2146$) and significantly higher in SPCs. VJ species were significantly higher in N and P (Table 4). The highest initial contribution of CWM SPCs in KO came from grasses (16.206), then *Salix* (12.225) and forbs (4.118). In VJ most CWM SPCs

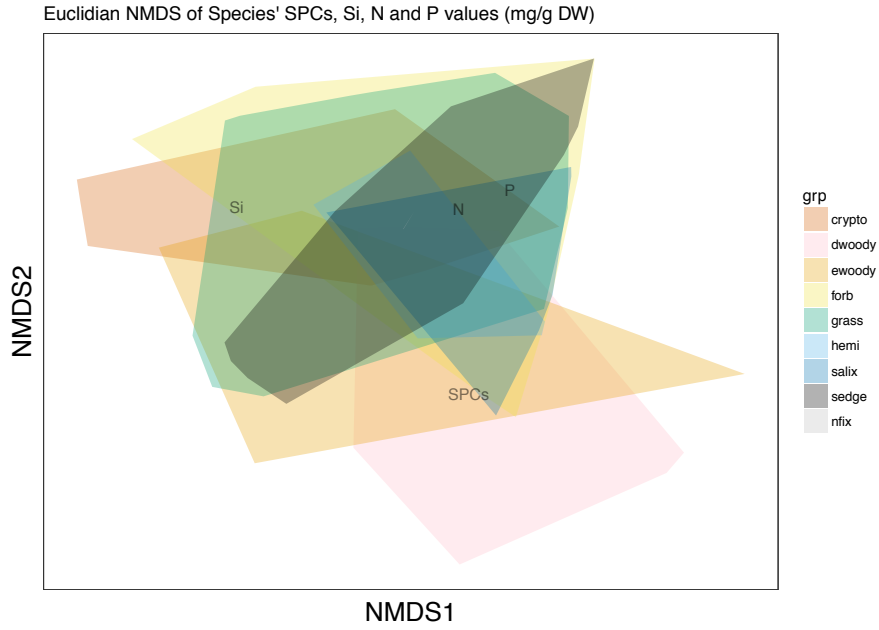


Figure 3: Ordination of species vs. SPCs, Silicon, and Nitrogen/Phosphorus with functional groups overlaid.

Table 1: LME correlation of SPCs, Silica and N

	<i>Dependent variable:</i>		
	SPCs	Si	N
N	-4.495*** (1.063)		
SPCs		-0.006*** (0.002)	
Si			-0.129*** (0.033)
Intercept	44.912*** (7.114)	1.249*** (0.115)	2.443*** (0.292)
Observations	635	635	635

Note: *p<0.1; **p<0.05; ***p<0.01

came from forbs (8.79, $p < 0.05$), followed by grasses (7.968, $p < 0.01$) and *Salix* (5.195, $p < 0.01$) (Table 5).

Functional group contributions of CWM Si in KO followed the same order as SPCs in KO, i.e.: grass (17.142) > *Salix* (8.720) > forb (4.366). In VJ differences were found with grass contributing the most (8.538, $p < 0.1$), then forb (8.25, $p < 0.1$) and finally *Salix* (8.720) (Table 6).

For N in KO, the greatest initial contributions came from grass (16.978), then *Salix* (7.661), then forbs (4.118). In VJ the greatest initial contributions came from grass (8.689, $p < 0.01$), then *Salix* (8.531), then forbs (8.384, $p < 0.05$).

NMDS ordination using the Bray-Curtis dissimilarity measure of CWM chemicals separated catchments into two *initially* (2006) significantly different regions ($R^2 = 0.133$, $p < 0.001$); KO defined by higher CWM- SPCs and Si, and VJ defined by high CWM- N/P (Plot A, Fig. 4). By 2008, however, differences between CWM chemicals appeared to lose the power to explain differences between catchments ($R^2 = 0.0142$, $p < 0.072$), though this finding is not quite significant (Table 2).

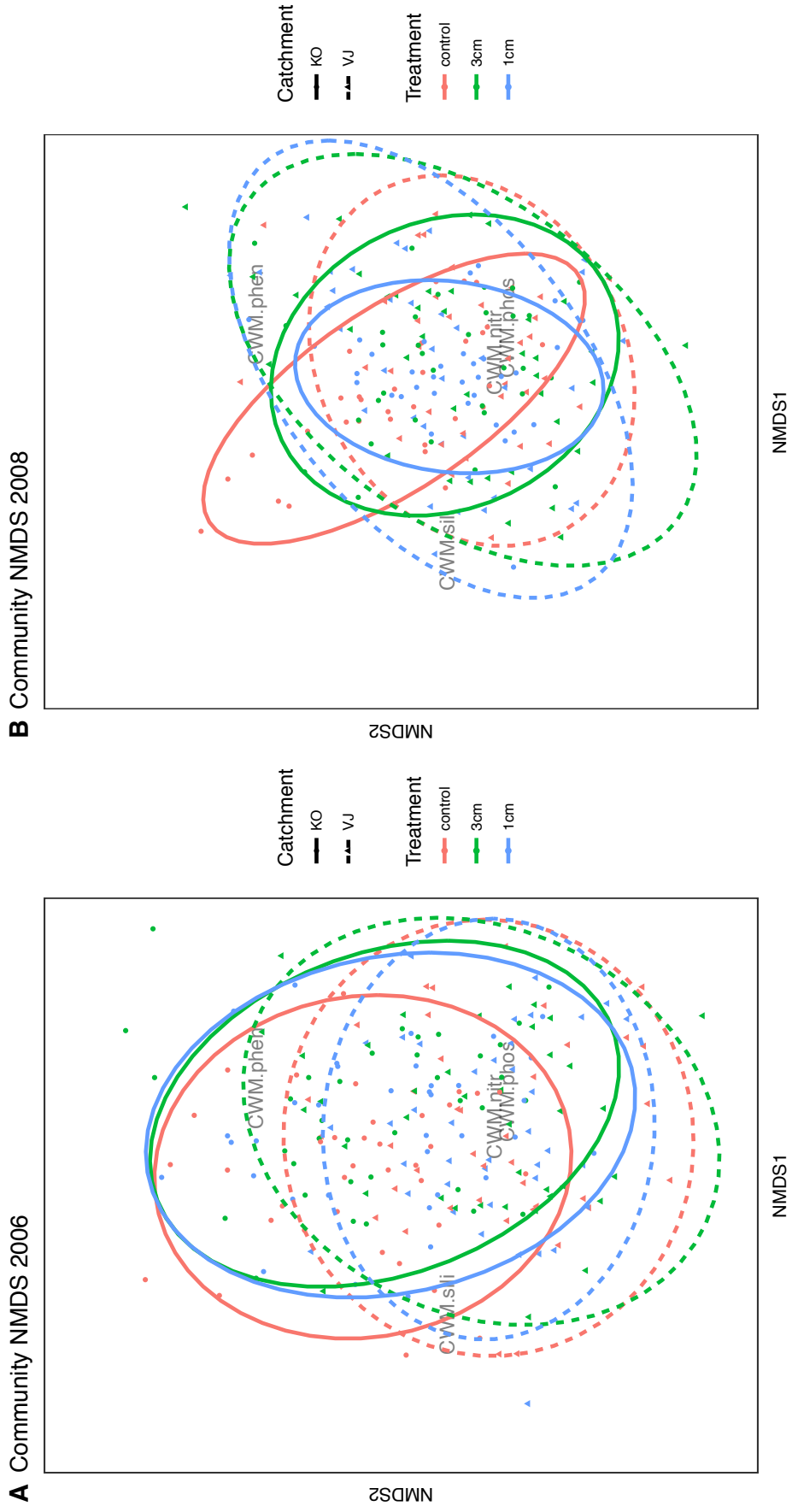


Figure 4: NMDS ordination of CWM- SPCs, Si, N and P profiles of catchment and treatment by year. In 2006, treatment already begins to separate community CWM chemical profiles. In 2008, KO is much more similar to VJ, and is better defined by tolerance traits than in 2006.

Table 2: Pairwise adonis statistics for catchment and year

	pairs	F.Model	R2	p.value	p.adjusted	sig
1	VJ:2006 vs KO:2006	34.26	0.13	0.00	0.01	*
2	VJ:2008 vs KO:2008	3.00	0.01	0.08	0.08	

Table 3: Pairwise adonis statistics for treatment and catchment

	pairs	F.Model	R2	p.value	p.adjusted	sig
1	VJ:2006:k vs VJ:2008:k	0.86	0.01	0.35	1.00	
2	VJ:2006:g vs VJ:2008:g	0.33	0.00	0.65	1.00	
3	VJ:2006:s vs VJ:2008:s	7.32	0.09	0.01	0.31	
4	KO:2006:k vs KO:2008:k	3.51	0.05	0.06	1.00	
5	KO:2006:g vs KO:2008:g	6.45	0.09	0.01	0.43	
6	KO:2006:s vs KO:2008:s	10.48	0.13	0.00	0.07	
7	VJ:2006:k vs KO:2006:k	16.35	0.18	0.00	0.07	
8	VJ:2006:k vs KO:2008:k	7.95	0.10	0.00	0.07	

Exclosure experiments

Already in 2006, herbivore exclosures significantly changed CWM chemical values within catchments; changing CWM- Si, N and P in KO, and CWM- SPCs and Si in VJ. Moreover, these changes were opposite between catchments. In KO, Si significantly decreased in 3cm (-0.151, $p < 0.01$) and decreased in 1cm (-0.103, $p < 0.1$) treatments, while N/P significantly increased. An insignificant decrease was also seen in SPCs for 1cm treatments. VJ by contrast significantly increased in both SPCs and Si in both treatments (Table 4). However adonis analysis of NMDS ordination groups showed CWM chemical values had no explanatory power for (within catchment) treatment differences in 2006 (KO: $R^2 = 0.00349$, $p = 0.905$; VJ: $R^2 = 0.0242$, $p = 0.241$).

In 2008, further changes are seen in VJ between treatments, with SPCs increasing in 3cm treatments (2.106), and significantly higher in 1cm treatments (9.497, $p < 0.05$) (Table 4). Although other CWM chemical changes are insignificant, the explanatory power of CWM chemicals for (within catchment) treatment differences in NMDS ordination appears to increase (KO: $R^2 = 0.03814$, $p < 0.133$; VJ : $R^2 = 0.042$, $p < 0.077$).

Adonis analysis also showed that changes in the same treatment between years in KO were significantly explained by treatment, and that the 1cm exclosures ($R^2 = 0.134$, $p = 0.004$) were better explained than 3cm exclosures ($R^2 = 0.0878$, $p = 0.005$) (Table 3; lines: 5 and 6). For VJ, only changes in 1cm mesh from 2006 to 2008 were significantly explained

by treatment ($R^2=0.0890$, $p=0.006$) (Table 3; line 3).

The control KO also moved towards VJ's original position with 18% of the difference between KO and VJ explained by grouping in 2006, but only 10.5% explain in 2008 (Table 3; lines: 7 and 8).

Table 4: LME correlation of SPCs, Silica and N

	<i>Dependent variable:</i>			
	SPCs	Si	N	P
Intercept	35.379*** (30.984, 39.774)	0.734*** (0.579, 0.889)	1.936*** (1.822, 2.051)	0.187*** (0.175, 0.200)
3cm Mesh	1.033 (-3.599, 5.665)	-0.210*** (-0.355, -0.066)	0.019 (-0.119, 0.157)	0.020** (0.004, 0.035)
1cm Mesh	0.927 (-3.704, 5.559)	-0.126* (-0.270, 0.018)	0.152** (0.014, 0.290)	0.022*** (0.006, 0.037)
VJ	-9.733*** (-15.828, -3.638)	-0.111 (-0.326, 0.104)	0.340*** (0.181, 0.499)	0.041*** (0.023, 0.058)
2008	-5.102** (-9.927, -0.277)	-0.039 (-0.189, 0.112)	-0.035 (-0.179, 0.108)	0.003 (-0.013, 0.019)
3cm Mesh:VJ	3.014 (-3.409, 9.437)	0.225** (0.025, 0.426)	0.073 (-0.118, 0.264)	0.0005 (-0.021, 0.022)
1cm Mesh:VJ	0.041 (-6.382, 6.464)	0.193* (-0.007, 0.393)	-0.084 (-0.275, 0.107)	-0.008 (-0.030, 0.013)
3cm Mesh:2008	-2.164 (-8.923, 4.595)	0.126 (-0.085, 0.336)	0.115 (-0.086, 0.316)	0.017 (-0.006, 0.039)
1cm Mesh:2008	-4.228 (-10.968, 2.512)	0.050 (-0.160, 0.260)	-0.017 (-0.217, 0.184)	0.007 (-0.016, 0.029)
VJ:2008	7.332** (0.703, 13.961)	-0.028 (-0.234, 0.179)	-0.082 (-0.279, 0.115)	-0.008 (-0.030, 0.014)
behandlings:hoveddaVJ:ar2008	2.106 (-7.191, 11.402)	-0.036 (-0.325, 0.254)	-0.039 (-0.315, 0.238)	-0.011 (-0.042, 0.020)
behandlings:hoveddaVJ:ar2008	9.497** (0.215, 18.780)	0.031 (-0.258, 0.320)	0.123 (-0.153, 0.399)	-0.002 (-0.033, 0.029)
Observations	435	435	435	435

Note:

*p<0.1; **p<0.05; ***p<0.01

4 Conclusion

NIRS Phenolic Measures

The separation of functional groups in this study into those investing primarily in SPCs forms of defense and those investing primarily in silica forms of defense fits PDS models. The strategies, although divergent, are best thought of as both inhabiting the “Nutrition and defense” region of figure 1.

According to defense theories presented by Endara and Coley (2011), constitutive defenses should be highest in species with long leaf lifetimes, i.e. evergreen species. That this study found the highest level of SPCs in woody deciduous species does not necessarily contradict defense theories if one assumes that evergreen species keep unpalatable leaves, a kind of mechanical defense—and indeed evergreen species have the lowest leaf nitrogen content of the functional groups studied (Fig. 7). Also figure 3 shows that species can have both high defenses and low nutrients. Defensive theories presented by Coley (1988); Endara and Coley (2011) also predict that the lowest constitutive defenses will be found in short-lived and grazing tolerant species, since energy is diverted towards growth. This is indeed the case for forbs and graminoids respectively. Although not stated in defense theories, but predicted here, graminoids and cryptograms should be even lower in SPCs than forbs since a portion of their energy budget is also dedicated to Si defenses, which act as constitutive defenses of another class, and for which trade-offs between carbon defenses have been found (Cooke & Leishman, 2012; Frew et al., 2016). *Deschampsia cespitosa* seems to be an exception to this rule, behaving much more defensively than its status as a grass should predict, and is indicative of the ever continuing process of evolution.

SPCs measures for hemiparasites not easily interpreted from available data. High foliar nitrogen content and low SPCs may mean they occupy a similar defense strategy as forbs, and as hemiparasites they conceivably face less resource constrictions that would limit regrowth. However, the genres within this functional group (*Bartsia*, *Euphrasia*, *Melampyrum* and *Pedicularis*) all belong to the family *Orobanchaceae* (ex-*Scrophulariaceae*), which has a low selectivity in the diet of grey-sided and tundra voles (Soininen et al., 2013). This may be indicative of other defensive strategies, namely alkaloids which are common in the *Scrophulariaceae* family and which *Orobanchaceae* is known to uptake from its

hosts (Lehtonen et al., 2005; Schneider & Stermitz, 1990).

Conclusions about the nitrogen fixing groups are also difficult to reach and may be due to the small sample size. Unfortunately in this study only one species, *Vicia cracca*, was included in this group; and of this species only 3 individuals sampled. In this study *V. cracca* was, despite predictions otherwise, found to have relatively high levels of SPCs and Si; while in other studies it was found to have low levels of phenolics, and score high in diet selectivity by *Microtus pennsylvanicus* (Bergeron & Jodoin, 1987). It is unknown whether these differences are due to sampling error, weak NIRS calibrations for this species, or local varieties revealing different strategies to different herbivores and climate.

Community Measures

KO and VJ significantly differed in their initial CMW SPCs, Si, N and P content. KO was significantly higher in both SPCs and Si, while VJ was significantly higher in N and P. This suggests species in each community have converged on syndromes that maximize fitness given different (a)biotic features—“Nutrition defense” in KO, “Tolerance/escape” in VJ. Soil N and P are known to be higher in VJ (Bråthen, K.A., personal communication, Nov. 2017).

The different responses to herbivore exclosure between KO and VJ found by (Ravolainen et al., 2011) are therefore suggested to be caused by different syndrome responses to trade-offs that occur within them due to herbivore removal.

However, from the data it appears that non-treatment related determinates are more influential on community composition than treatment effects in these two systems. This suggests then, that the duration of such herbivory exclosure experiments must be lengthened before effects on CMW SPCs due to herbivory become statistically significant against the backdrop of, much larger, seasonal changes; and the changes that herbivore cycles would correspondingly elicit in plant communities.

However, some changes are apparent within this 3 year period. In VJ for example, herbivore exclosures favour *Salix* at the expense of grasses. This raises total CWM SPCs in this community since *Salix* is particularly high in SPCs. That grasses decrease in biomass is a reflection of their tolerance traits, including symbiosis, with herbivores, and the removal of their herbivore symbionts also removes their ability to apparently compete with other plant species, namely *Salix* also favoured by reindeer, and inhabiting a

defense/growth region. Sedges increased with the removal of herbivores, as well, because their strategy is more defensive/growth oriented and less tolerant than their graminoid counterparts, and indeed they have more SPCs and Si than grass. In KO, grasses also seemed to trade-off in enclosure treatments; their biomass remaining statistically unchanged while forbs increased.

In KO CWM SPCs decreased in grasses, seemingly due to the replacement of *Deschampsia cespitosa*, a grass relatively rich in SPCs, with *Avenella flexuosa*, a grass relatively poor in SPCs. That total CWM SPCs in KO decreased with treatment, therefore, seems to be related to the non-treatment related decrease in *Salix*, and the replacement of a SPCs rich grass with a SPCs poor grass. That *D. cespitosa* and *A. flexuosa* are not only respectively high and low in SPCs, but high and low in silica strengthens trade-offs in energy allocation, and is probably why the change between these two species is so apparent.

For the same study system Ravolainen et al. (2011) concluded that the differing responses between KO and VJ were attributable to herbivore loads and the initial composition of vegetation; and that in KO *D. cespitosa*, a stated high silica grass, was replaced by *A. flexuosa*, a stated low silica grass. This study furthers those conclusions by showing that not only was KO marked by an initial composition high in silica rich grass species (*D. cespitosa*), but that the entire community was marked by higher levels of both silica and SPCs. Initial conditions in KO were, therefore, in a highly defended state, that upon release quickly moved towards a less defense oriented community.

In VJ by contrast, initial conditions (probably higher resource levels) already favoured a community state low in defense. Defensive trade-offs being low, the community simply grew in response to herbivore release. It appears this release was unfavourable to a palatable, perhaps more grazing tolerant assemblage of grasses, which decreased in abundance, being crowded out by much larger and phenolic rich, *Salix*; which, although being heavily defended, is a palatable species with a high variability in its phenolic content and overlap into N/P regions.

Community reactions to herbivore enclosure and other (a)biotic conditions (indicated by the increase of forbs in both catchments between years), appear to drive both catchments towards a new and more similar optimum; and that this effect appears to be

strongest for KO, which is the least resource rich of the two catchments. The interplay, then, between defense-, growth- and tolerance oriented species, their herbivores and abiotic conditions supports the assertion that current plant defense theories lack an accurate accounting of possible plant defensive strategies; and is in agreement with the plant defense syndromes (PDS) model proposed by Agrawal and Fishbein (2006). Furthermore, figure 4 suggests that trade-offs at higher scales can be directly modeled from the discovery of trade-offs at lower scales. The discovery of trade-offs in defense strategy among species, serves as the backdrop to model syndrome trade-offs in communities moving towards optimums. Visually, Plot ‘A’ of figure 4 shows two communities in which (a)biotic factors have conspired to create two different energy use optimums. Removal of herbivores released constraints on those community optimums, and is indicated by the separation of the control, 3-, and 1cm treatments in both catchments. Plot ‘B’ of figure 4 begins to model community changes through time. In this example, combined (a)biotic forces have moved the energy use optimum of KO towards that of VJ, and (somewhat) *vice versa*—it appears KO had a stronger response than VJ. It is not hard to imagine that a new optimum is reached in both communities, forcing species to adapt; and over evolutionary time, converge on new syndrome suites. It is also not hard to imagine the model in a reverse temporal dimension in which the continued force of (a)biotic drivers pushes the two communities further apart, forcing energy allocation towards new optimums, and ultimately driving speciation over evolutionary time periods.

However, abiotic forces are changing, and biotic changes are adaptive; precluding (or at least highly complicating) predictive power. Evidence such as: (1) the palatability of *Salix* despite high SPCs; (2) the variability of SPCs among related *Salix*; (3) syndrome differences between (the phylogenically related) *D. cespitosa* and *A. flexuosa*; (4) secondary defense chemicals being beneficial (Smilanich et al., 2016); and (5) defensive strategies in *Asclepias spp.* being ineffective against a specialist herbivores (Agrawal & Fishbein, 2006), must be considered in the temporal perspective in which the studies occurred (i.e. a near instant in the continuing co-evolution of plant/herbivore interactions).

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	<i>Dependent variable:</i>					
	dwoody	salix	forb	sedge	grass	crypto
Constant	1.327 (-0.386, 3.041)	12.225*** (8.455, 15.994)	4.118*** (1.429, 6.806)	0.847** (0.110, 1.584)	16.206*** (14.208, 18.203)	0.241 (-0.383, 0.865)
behandlings	0.722 (-1.332, 2.776)	0.927 (-3.375, 5.230)	0.069 (-2.421, 2.558)	-0.497 (-1.089, 0.094)	-0.298 (-2.213, 1.617)	0.047 (-0.519, 0.614)
behandlings	1.207 (-0.845, 3.260)	0.155 (-4.145, 4.455)	0.585 (-1.904, 3.073)	-0.765** (-1.356, -0.173)	-1.885* (-3.798, 0.029)	-0.117 (-0.683, 0.449)
hoveddalVJ	-0.529 (-2.726, 1.667)	-7.030*** (-11.901, -2.159)	4.672** (1.109, 8.235)	0.423 (-0.564, 1.411)	-8.238*** (-10.876, -5.600)	0.400 (-0.429, 1.228)
ar2008	-0.249 (-2.227, 1.728)	-8.801*** (-12.944, -4.658)	0.800 (-1.598, 3.198)	-0.542* (-1.112, 0.028)	1.968** (0.123, 3.812)	0.159 (-0.387, 0.704)
behandlings:hoveddalVJ	-0.519 (-2.893, 1.855)	2.739 (-2.235, 7.713)	0.759 (-2.119, 3.638)	0.061 (-0.623, 0.745)	-0.476 (-2.690, 1.738)	0.702** (0.048, 1.357)
behandlings:hoveddalVJ	-1.610 (-3.981, 0.760)	2.738 (-2.229, 7.705)	0.131 (-2.744, 3.005)	0.176 (-0.507, 0.859)	1.506 (-0.705, 3.716)	0.759** (0.105, 1.413)
behandlings:ar2008	-0.453 (-2.826, 1.920)	0.549 (-4.422, 5.520)	0.759 (-2.118, 3.635)	0.203 (-0.481, 0.886)	-2.508** (-4.720, -0.295)	0.452 (-0.202, 1.107)
behandlings:ar2008	-0.527 (-2.896, 1.843)	2.297 (-2.667, 7.261)	0.249 (-2.624, 3.121)	0.649* (-0.034, 1.331)	-2.204* (-4.413, 0.006)	0.453 (-0.200, 1.107)
hoveddalVJ:ar2008	0.688 (-1.246, 2.621)	12.598*** (8.547, 16.648)	-1.666 (-4.010, 0.678)	0.425 (-0.132, 0.982)	-0.841 (-2.643, 0.962)	-0.382 (-0.915, 0.152)
Observations	435	435	435	435	435	435

Note:

*p<0.1; **p<0.05; ***p<0.01

Table 5: Functional Group LME Model of Community Weighted Mean Soluble Phenolics

	<i>Dependent variable:</i>					
	woody	salix	forb	sedge	grass	crypto
Constant	1.313* (-0.203, 2.830)	8.720*** (5.290, 12.150)	4.366*** (1.857, 6.875)	0.612* (-0.089, 1.312)	17.142*** (15.283, 19.002)	0.193 (-0.391, 0.777)
behandlingsg	0.505 (-1.215, 2.224)	1.070 (-2.698, 4.838)	0.440 (-1.649, 2.529)	-0.409 (-0.908, 0.089)	-1.463* (-3.078, 0.152)	0.262 (-0.215, 0.738)
behandlings	0.950 (-0.764, 2.665)	1.085 (-2.672, 4.843)	0.719 (-1.364, 2.802)	-0.463* (-0.960, 0.034)	-2.903*** (-4.514, -1.292)	0.101 (-0.375, 0.576)
hoveddaIVJ	-0.201 (-2.200, 1.798)	-1.138 (-5.675, 3.399)	3.884** (0.496, 7.272)	0.617 (-0.336, 1.569)	-8.604*** (-11.108, -6.099)	0.215 (-0.574, 1.004)
ar2008	-0.217 (-1.180, 0.745)	-1.194 (-3.303, 0.914)	0.260 (-0.909, 1.429)	-0.030 (-0.309, 0.249)	-0.065 (-0.969, 0.839)	0.263* (-0.004, 0.529)
behandlings:hoveddaIVJ	-0.516 (-2.883, 1.851)	2.942 (-2.244, 8.129)	0.746 (-2.129, 3.621)	0.071 (-0.615, 0.758)	-0.531 (-2.754, 1.692)	0.704** (0.048, 1.360)
behandlings:hoveddaVJ	-1.604 (-3.967, 0.759)	3.016 (-2.162, 8.195)	0.103 (-2.768, 2.974)	0.193 (-0.493, 0.878)	1.455 (-0.765, 3.675)	0.758** (0.103, 1.413)
Observations	435	435	435	435	435	435

Note:

*p<0.1; **p<0.05; ***p<0.01

Table 6: Functional Group LME Model of Community Weighted Mean Silicon

	<i>Dependent variable:</i>					
	dwoody	salix	forb	sedge	grass	crypto
Constant	1.691** (0.359, 3.022)	7.661*** (4.626, 10.696)	4.215*** (1.868, 6.562)	0.565* (-0.103, 1.232)	16.978*** (15.246, 18.709)	-0.067 (-0.614, 0.480)
behandlingsg	0.232 (-0.950, 1.413)	2.624** (0.035, 5.212)	0.834 (-0.599, 2.266)	-0.371** (-0.713, -0.029)	-1.742*** (-2.854, -0.631)	0.634*** (0.304, 0.963)
behandlings	0.106 (-1.073, 1.286)	2.675** (0.090, 5.260)	0.774 (-0.656, 2.205)	-0.362** (-0.703, -0.020)	-2.139*** (-3.250, -1.029)	0.500*** (0.171, 0.829)
hoveddalVJ	-0.917 (-2.365, 0.531)	0.870 (-2.507, 4.247)	4.169*** (1.227, 7.112)	0.706 (-0.158, 1.570)	-8.289*** (-10.434, -6.145)	0.708* (0.021, 1.396)
ar2008	-0.214 (-1.176, 0.748)	-1.199 (-3.306, 0.909)	0.260 (-0.906, 1.427)	-0.030 (-0.309, 0.248)	-0.068 (-0.974, 0.837)	0.261* (-0.007, 0.530)
Observations	435	435	435	435	435	435

Note: *p<0.1; **p<0.05; ***p<0.01

Table 7: Functional Group LME Model of Community Weighted Mean Nitrogen

Plant group	b	n
Narrow leaved grasses	6.29	25.00
Broad leaved grasses	8.90	20.00
Cyperaceae	11.30	19.00
Large forbs	15.34	14.00
Medium sized forbs	13.52	47.00
Small forbs	6.94	21.00
Evergreen ericoids, leaves only	60.90	17.00
Evergreen ericoids	82.50	16.00
Broad leaved deciduous shrubs, incl. Salix leaves only	15.80	25.00
Broad leaved deciduous shrubs, incl. Salix	33.31	24.00
Betula nana, leaves only	19.80	15.00

Table 8: Biomass conversion factors for species occurring in the KO and VJ study area. Point intercept frequency count is divided by 3 to get average per pin per plot, then multiplied by the relevant b-value for conversion to grams per plot and divided by 0.21 (grid size: $0.42m \times 0.5m$) to get grams m^{-2}

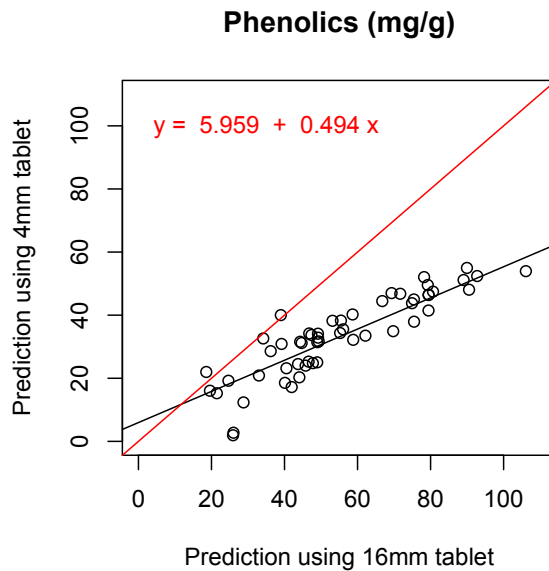


Figure 6: NIRS correction factor from 4mm to 16mm adapter. From TOMASSI project.