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# The NutNet experiment: preliminary results from the swiss site after one year.

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## Abstract

Today's anthropogenic impacts on terrestrial ecosystems are manifold and profound. Regarding grasslands, nutrient deposition and removal of herbivores are among the most crucial consequences of human activity. The global NutNet initiative aims to improve the understanding of their effects on grasslands on a global scale. Applied treatments include herbivore exclosure and fertilization with N, P and K<sup>+</sup> (including micronutrients) in a full-factorial design, while measuring productivity, PFT abundance and species cover. Presented data cover the alpine swiss NutNet site before and after one year of treatment application. While no effect on the community biomass was visible in an ANOVA, fertilization with N influenced the competitive relationship between graminoids and forbs by increasing the abundance of forbs, but only in presence of additional K and micronutrients. Unexpectedly, herbivore exclosure had no significant effect on productivity neither at community scale nor at the scale of PFTs. Also no signal was detected on the level of species cover. The same was true for a test of the diversity-productivity hypothesis.

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# 1. Introduction

Mankind today has profound impact on nature, including grasslands. Beside the well-debated changing climate, nutrient inputs are among the major ways of human influence (e.g. Stevens et al. 2004). In addition, mankind has major impact in naturally occurring herbivores by removing species via hunting, habitat degradation etc., but also by introducing new species. Despite the fact of these global human impacts, their effect on grasslands are to date not well understood. The NutNet initiative aims to analyse impacts of nutrient deposition and changes in herbivory pressure on a global scale. To date, the initiative includes over 40 plots, located on five continents in North and South America, Europe, Africa, Asia and Australia. The methodological approach is the same on each site, allowing comparisons on a global scale. In addition to the impacts of nutrients and herbivores, the NutNet design allows testing the relation between species richness and productivity in grasslands. After the BIODEPTH project analysed this on a European scale (Hector et al. 1999), the NutNet initiative has now even the opportunity to test the relation at a global scale. Thereby it will potentially contribute to the understanding of the conditions, under which the diversity-productivity hypothesis holds true. This is of major importance facing today's high extinction rates of species.

While most NutNet sites are located in the lowlands and relatively productive, the Swiss site is a low productive alpine grassland, which potentially reacts differently than the other sites and is thus of particular interest. The work at hand presents results from a preliminary analysis of the Swiss site after one year of treatment application. The explicit questions (Q) and hypotheses (H) are the following.

H1: There is a positive relationship between diversity and productivity on the plots.

Q1: What effects does fertilization with N, P and K have on vascular plant productivity and the abundances of the different plant functional types (PFTs)?

Q2: Does the exclosure of large herbivores effect vascular plant biomass and the abundances of the different PFTs?

H2: The treatments lead to changes in the vegetation pattern.

The different hypotheses and questions will be tested/answered in the following.

## 2. Methods

### 2.1 Experimental design & data collection

#### THE SITE

The Swiss site of the NutNet is located above the village Lü in the Münster valley in the cantone of Grisons. It is situated at an elevation of approx. 2360 m a. s. l. on a relatively steep ( $\sim 30^\circ$ ) southwest facing slope amid avalanche barriers. The vegetation can be seen as a typical low-productive dry alpine grassland and is only grazed by naturally occurring herbivores, mainly ibex (*Capra ibex*), alpine marmot (*Marmota marmota*), and alpine mountain hare (*Lepus timidus varronis*). Three experimental blocks were established in 2008. They are located at approximately same altitude along the slope within about 200m. The three blocks each contain 10 plots of a size of 5 x 5 m, which in turn are divided into 4 subplots of 2.5 x 2.5 m (A' to D'). These again are subdivided into 4 quadrates (1 to 4) of 1 x 1 m (FIGURE 1).

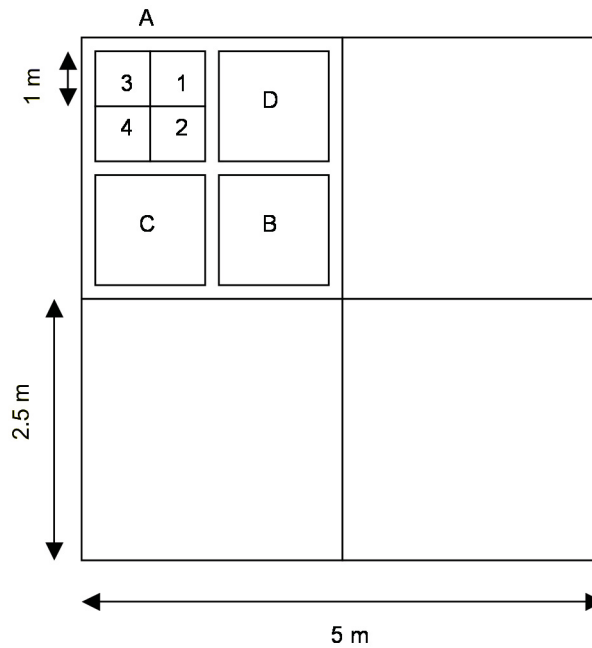


FIGURE 1. Arrangement of subplots and quadrates using the example of plot 6 in block 3.

#### TREATMENTS

The treatments follow a completely randomized block design. Three different nutrient treatments (N, P and K+) were applied in a full-factorial design, with two levels per factor (control, added).

N: Nitrogen was added in the form of urea ( $\text{N}_2\text{H}_4\text{CO}$ ), at a rate of  $23.3 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ , which works out to  $10 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of N.

P: Phosphorous was given as triple super phosphate  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  at a rate of  $50.9 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ , which works out to  $10 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of P,  $8.1 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of Ca and traces of S and Mg.

K+: this treatment included fertilization with potassium sulphate ( $K_2SO_4$ ) at  $22.3 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  and Scotts micromax at  $100 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ . This works out to  $10 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of K,  $17 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of Fe,  $15.9 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of S,  $6.0 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of Ca,  $3.0 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of Mg,  $2.5 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of Mn,  $1.0 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of each Cu and Zn and  $0.1 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$  of each B and Mn.

The full factorial design led to 8 different nutrient treatments, applied on the plots 1 – 8 (cf. TABLE 1).

Additionally to the nutrient treatments, two plots per block were fenced to prevent grazing by large (ibex) and medium sized (marmot, hare) mammalian herbivores. Common wire-mesh fences of about 2 m height were used, stabilized by wooden poles. The fences had a distance of 1 m to the boundary of the inside plots. Plot 9 included no fertilization and exclosure of herbivores, treatment of plot 10 was exclosure and application of all nutrients (cf. TABLE 1). The treatments were first applied in early summer 2009.

TABLE 1. The different treatments of the plots: fertilization using nitrogen (N), phosphorous (P), potassium and others (K+) and exclosure of large herbivores using a fence. The treatments were replicated in three different blocks ( $n = 3$ ).

	N	P	K+	Exclosure
Plot 1	0	0	0	0
Plot 2	0	0	1	0
Plot 3	0	1	0	0
Plot 4	1	0	0	0
Plot 5	0	1	1	0
Plot 6	1	0	1	0
Plot 7	1	1	0	0
Plot 8	1	1	1	0
Plot 9	0	0	0	1
Plot 10	1	1	1	1

#### DATA COLLECTION

The data used here are all originating from the same  $2.5 \times 2.5 \text{ m}$  subplot unit (the one called ,A'). Vegetation data was sampled according to the method of Braun-Blanquet (1964) both in 2008 and 2009 in quadrat 1. Productivity data was gained in late summer 2008 and 2009 in quadrat 3 by clipping all aboveground biomass at ground level in two  $1 \times 0.1 \text{ m}$  stripes per the  $1 \times 1 \text{ m}$  quadrat. Different stripes (in the same  $1 \times 1 \text{ m}$  quadrat) were used in 2008 and 2009, respectively.

The clipped biomass was sorted manually into the plant functional types (PFTs) of graminoids, non-leguminous forbs (,forbs'), legumes, woody species and dead material produced in the previous year. The dead material was excluded from the further analyses, in order to have only biomass produced in the current year. The NutNet design also foresaw the separation of bryophytes, but non were present on our plots. The division between woody species and forbs was somewhat unclear, because many occurring species had at least some woody parts. It was decided to take only *Vaccinium vitis-idea*, *Daphne striata*, *Juniperus communis* into the ,woody section'.

After being divided into different groups, the samples were dried in the oven for 48 h at 60°C and weighed to the nearest 0.01g thereafter.

## 2.2 Data analysis

All analyses were conducted using the R software, version 2.9.2. If necessary, the data was transformed using „first aid“ transformations (Mosteller and Tukey, 1977):

$$\begin{array}{ll} y' = \log(y) & \text{for total biomass values } (y \geq 0) \\ y' = \arcsin(\sqrt{y}) & \text{for abundances } (1 \geq y \geq 0) \end{array}$$

In the following, the methods used to assess the different hypotheses and questions are presented.

H1: THERE IS A POSITIVE RELATIONSHIP BETWEEN DIVERSITY AND PRODUCTIVITY ON THE PLOTS.

To test this hypothesis, the number of vascular plant species were used. To avoid effects of the treatments, only data gained in 2008 were included. A linear regression was run to test whether there is a positive relationship between the number of vascular plant species per plot and the total biomass clipped in late summer 2008 (not transformed), before the start of the fertilizer treatments. Also, a polynomial regression including the linear and the quadratic term of the number of vascular plants was run to test whether a hump-shaped curve could be fitted to the data.

Q1: WHAT EFFECTS DOES FERTILIZATION WITH N, P AND K HAVE ON VASCULAR PLANT PRODUCTIVITY AND THE ABUNDANCES OF THE DIFFERENT PFTs?

This was tested in two ways. Both of them are using the total biomass and the abundances of graminoids, forbs, legumes and woody species as target variables. Firstly, a two-way ANOVA was run for each target variable, using data gained in late summer 2009. First aid transformations were applied to all target variables. Explanatory factors of two levels each were the application of N, P and K+ fertilizer. The different blocks (1-3) were included as an error term. Using this method, the more stronger the effect of a treatment is, compared to the existing (spatial) variability within the blocks, the more likely it is to get a significant result.

The second way focuses on single plots. It implies comparing the values of 2009, which were gained after having treated the plots, against the observations done in late summer 2008, before the treatments. Given the effects of the treatments are stronger than the inter annual variation between 2008 and 2009, systematic changes in the target variables should be visible, depending on the treatment of each plot. This was tested using ANOVAs separately for each target variable.

**Q2: DOES THE EXCLOSURE OF LARGE HERBIVORES EFFECT VASCULAR PLANT BIOMASS AND THE ABUNDANCES OF THE DIFFERENT PFTs?**

This was tested using one-way ANOVAs for the target variables total biomass and abundances of PFTs, using herbivore exclosure as the only factor (of two levels). To avoid additional effects of the other treatments (and an unbalanced data set), these tests were carried out using a subset including only plot 1, 8, 9 and 10. These plots were treated with no or all fertilization treatments, with plot 1 & 8 being unfenced and plot 9 & 10 being fenced (cf. TABLE 1). Fertilizer application was used as an error term, to ensure that the only systematic difference between the compared plots is the absence or presence of herbivore fence. First aid transformations were applied where they resulted in a higher p-value of a shapiro-wilk-test of normality (Shapiro and Wilk, 1965).

**H2: THE TREATMENTS LEAD TO CHANGES IN THE VEGETATION PATTERN.**

To test this hypothesis, cover data (ranging from 0 to 1) of 92 species gained by in late summer 2009 was used. To detect vegetation patterns, a cluster analysis was run using an agglomerative approach. Several distance types and linkages were tried out to find to best separation. Finally, manhattan distances and complete linkage were chosen.

### **3. Results**

**H1: THERE IS A POSITIVE RELATIONSHIP BETWEEN DIVERSITY AND PRODUCTIVITY ON THE PLOTS.**

FIGURE 2 shows the relation between aboveground productivity and diversity on the 30 plots in 2008. Apparently, at this scale, there is no relationship. This was confirmed when running a linear regression ( $p = 0.809$ ). It was also tried to fit a quadratic relation using polynomial regression. This led to a slightly lower, though by far not significant p-value (from the overall F-statistics):  $p = 0.4231$ . A closer look at FIGURE 2 might suggest that the exclusion of block 3 could lead to a (more) significant result of the linear regression, as block 3 shows higher species diversity but not higher productivity. This was tried and also the log-transformation of the dependent variable. The lowest p-value ( $p = 0.1607$ ) was gained in a linear regression using log-transformation and exclusion of block 3.



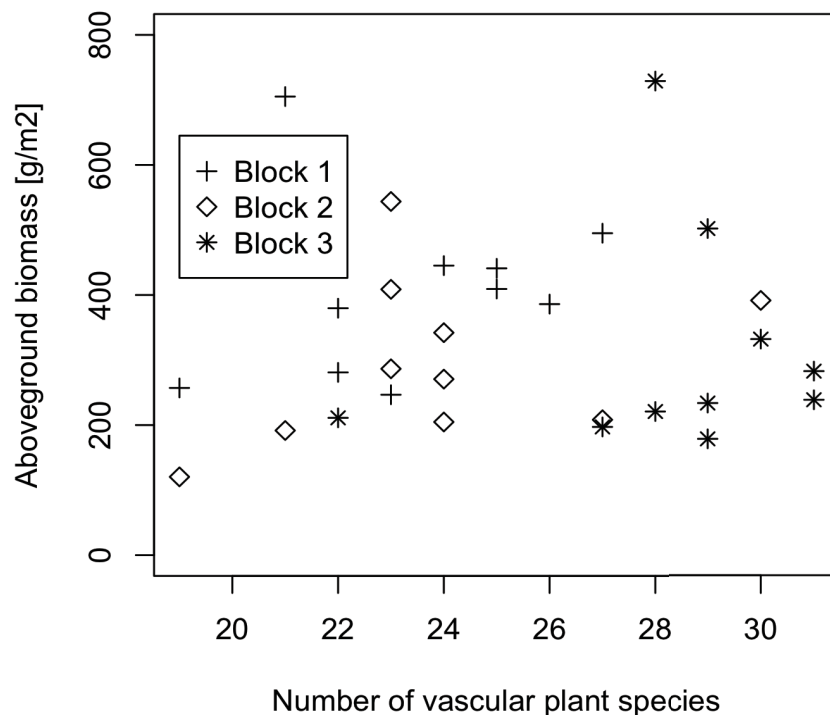


FIGURE 2. Diversity of vascular plant species and aboveground biomass clipped in late summer 2008 on the 30 plots. The different blocks are symbolized by different symbols.

Q1: WHAT EFFECTS DOES FERTILIZATION WITH N, P AND K HAVE ON VASCULAR PLANT PRODUCTIVITY AND THE ABUNDANCES OF THE DIFFERENT PFTs?

As stated above, two ways were used to try to answer this question. As a result of the first method, TABLE 2 shows the means of the overall aboveground biomass at harvest in late summer 2009 and the abundances of the different PFTs. Means and standard errors are calculated with  $n=12$  for both the „control“ and the „added“ level of each nutrient treatment factor. The shown p-values correspond to the probability of each factor in a two-way ANOVA run for each target separately. None of the differences in the shown means is significant. The values given in bold refer to potential trends ( $p<0.25$ ). Treating the plots with K and other nutrients might have had a positive effect on the total aboveground biomass of the plots. Besides that, the fertilization with nitrogen led to a ‚trend‘ in most PFT abundances. Adding nitrogen to the plots might decrease the abundance of graminoids, while increasing the abundance of forbs and woody species and having no effect on legumes. Meanwhile, the fertilization with P might decrease the abundance of woody species.

TABLE 2. Means and standard error of the total biomass in late summer 2009 in g\*m-2 and the abundance of the different PFTs as influenced by the three different nutrient treatments. The p-values refer to the probability of the corresponding factor in a blocked two-way ANOVA as described in the methods paragraph. Values given in bold if the corresponding p-value is <0.25.

Treatment		N		P		K+	
		control	added	control	added	control	added
Biomass [g*m <sup>-2</sup> ]	mean± s.e.	335.38±32.87	330.90±40.00	344.30±45.71	321.98±23.96	<b>307.66±40.08</b>	<b>358.62±31.34</b>
	p- value	0.8046		0.8027		<b>0.1230</b>	
	mean± s.e.	<b>0.592±0.059</b>	<b>0.487±0.063</b>	0.546±0.064	0.534±0.061	0.567±0.067	0.513±0.056
Graminoid abundance	p- value	<b>0.1183</b>		0.8652		0.5232	
	mean± s.e.	<b>0.379±0.059</b>	<b>0.466±0.063</b>	0.417±0.063	0.428±0.062	0.399±0.066	0.446±0.058
	p- value	<b>0.2140</b>		0.8926		0.6054	
Forb abundance	mean± s.e.	0.010±0.003	0.015±0.004	0.011±0.003	0.014±0.004	0.015±0.005	0.010±0.003
	p- value	0.3133		0.3683		0.3966	
	mean± s.e.	<b>0.005±0.004</b>	<b>0.021±0.010</b>	<b>0.018±0.009</b>	<b>0.008±0.006</b>	0.009±0.007	0.017±0.009
Woody species abundance	p- value	<b>0.1502</b>		<b>0.2233</b>		0.5676	

*Note:* The abundance might not sum up to 1.0 due to rounding errors. Interactions are not shown here, because all were *n.s.*

TABLE 3 shows the results of the analysis of the per-plot difference between 2009 and 2008. Differences greater zero indicate a higher value in 2009 compared to 2008. Additionally, higher values for the „added“ level of a factor compared to its „control“ level indicate positive effects of the factor on that value. As the treatments only started in 2009, the 2008 values can be seen as control data, thus, a significant p-value for a difference in a value indicates an effect of the corresponding factor on the variable itself.

The results show no significant effects on aboveground vascular plant biomass. The numbers indicate both a high inter annual variation on each plot (differences ≠ 0), as well as a high variation between the different plots (high standard errors).

In respect to the abundance of graminoids, TABLE 3 indicates a trend towards a negative effect of N fertilization. This is in line with the results shown in TABLE 2. Adding nitrogen to the plots seems to be decreasing the abundance of graminoids. Meanwhile, adding nitrogen to the plots significantly increases the abundance of non-leguminous forbs (p=0.0253), even though this effect interacts with the fertilization with potassium and other elements. The interaction is shown in FIGURE 3. Generally, forbs were more abundant in 2008, resulting in negative differences. Only in the plots fertilized with nitrogen *and* potassium and others, forbs showed a higher abundance in 2009. This effect is significant as indicated by the results of a TukeyHSD test (cf. FIGURE 3) and the results of an ANOVA using subsets, testing the positive effect of nitrogen fertilization on forbs when potassium and other elements are added (p = 0.0110), respectively not added (p = 0.8736).

TABLE 3. Differences in aboveground biomass and abundances of the different PFTs between 2009 and 2008, calculated as  $\text{value}_{2009} - \text{value}_{2008}$ . Thus, differences  $> 0$  indicate larger values in 2009 than in 2008. A higher value for the „added“ level of a factor compared to its „control“ level indicates a positive effect of the factor on that variable.

Treatment		N		P		K+	
		control	added	control	added	control	added
Difference in biomass [g·m <sup>-2</sup> ]	mean± s.e. p-value	-36.66 ±40.63	27.16±24.93	-10.88±42.40	1.38±24.79	0.58±26.63	-10.08±41.28
		0.2284		0.9921		0.6432	
Difference in graminoid abundance	mean± s.e. p-value	<b>0.167±0.028</b>	<b>0.074±0.033</b>	0.118±0.031	0.123±0.035	0.147±0.022	0.094±0.040
		<b>0.0524</b>		0.5973		0.3787	
Difference in forb abundance	mean± s.e. p-value	<b>-0.109±0.027</b>	<b>-0.015±0.030</b>	-0.060±0.028	-0.064±0.034	-0.093±0.022	-0.031±0.036
		<b>0.0253*, p(N:K)=0.0332*</b>		0.5464		0.2273	
Difference in legume abundance	mean± s.e. p-value	-0.029±0.011	-0.054±0.016	-0.038±0.011	-0.045±0.016	-0.041±0.011	-0.042±0.016
		0.1745		0.9357		0.8249	
Difference in woody species abundance	mean± s.e. p-value	0.005±0.004	0.009±0.011	<b>0.018±0.009</b>	<b>-0.003±0.005</b>	0.009±0.007	0.006±0.009
		0.7030		<b>0.0479*</b>		0.9810	

*Note:* In a one-way ANOVA, the p-values for the influence of N on difference in total biomass is significant on the 5% level ( $p = 0.0465^*$ ).

Interactions were included in the model, but not shown here because all were *n.s.*, except for N:K in the graminoids ANOVA ( $p = 0.0332^*$ ).

No transformations were applied, due to value range of the target variables, but shapiro-wilk-test was *n.s.* in all cases. For the ANOVA on the abundance of woody biomass, the shapiro-wilk-test was near significance ( $p = 0.056$ ), therefore the results have to be handled with care.

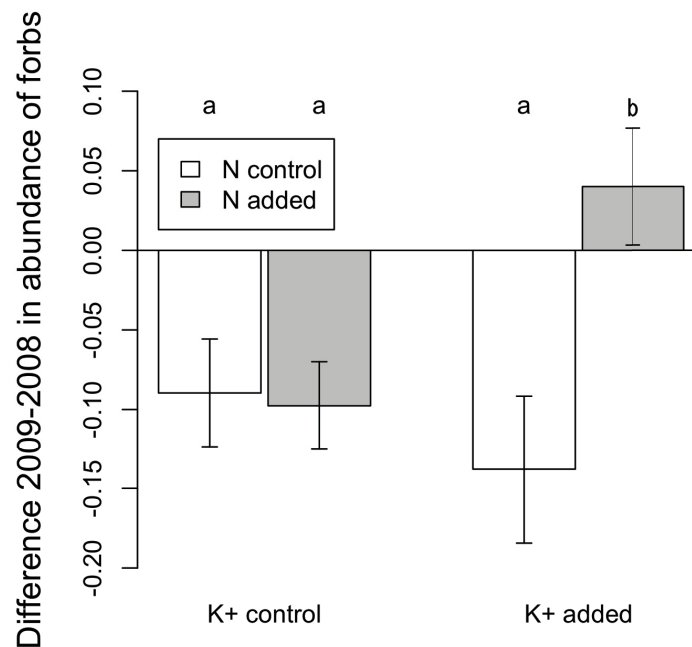


FIGURE 3. Difference 2009 – 2008 in abundance of non-leguminous forbs in dependence of nitrogen and potassium fertilization. The numbers above the bars relate to significant differences as gained by a TukeyHSD test on the 10% level (all p-values were  $< 0.08$ , some  $< 0.05$ ).

## Q2: DOES THE EXCLOSURE OF LARGE HERBIVORES EFFECT VASCULAR PLANT BIOMASS AND THE ABUNDANCES OF THE DIFFERENT PFTs?

As shown in TABLE 4, herbivore exclusion had no significant effect on the total clipped biomass in late summer 2009 nor on the abundances of the different PFTs. The p-values of the corresponding ANOVAs are too high to even discuss a potential trend, with the lowest being  $p = 0.4369$ . Thus, there is no support for our hypothesis that exclosure of large herbivores leads to a higher biomass on the plots.

TABLE 4. Effects of large herbivore exclosure on total biomass as clipped in late summer 2009 and abundances of the different PFTs in the plots 1, 8, 9 and 10. The p-values refer to a one-way ANOVA including fertilization treatments as an error term.

Herbivore exclosure		Applied	Control
Biomass [ $\text{g}\cdot\text{m}^{-2}$ ]	Mean $\pm$ s.e.	273.91 $\pm$ 34.33	334.43 $\pm$ 64.10
	p-value		0.4369
Graminoid abundance	mean $\pm$ s.e.	0.4785 $\pm$ 0.0870	0.5123 $\pm$ 0.1207
	p-value		0.7697 ‡
Forb abundance	mean $\pm$ s.e.	0.4941 $\pm$ 0.0823	0.4517 $\pm$ 0.1266
	p-value		0.7174 ‡
Legume abundance	mean $\pm$ s.e.	0.0140 $\pm$ 0.0060	0.01375 $\pm$ 0.0058
	p-value		0.9520 ‡
Woody species abundance	mean $\pm$ s.e.	0.0024 $\pm$ 0.0019	0.0163 $\pm$ 0.0163
	p-value		0.6530 ‡

Notes: ‡ Tukey first aid transformation applied. For the abundance of woody species, the shapiro-wilk-test still led to a significant result after applying first aid transformation. Thus, this number has to be handled with care.

## H2: THE TREATMENTS LEAD TO CHANGES IN THE VEGETATION PATTERN.

FIGURE 4 shows the result of the cluster analysis in a dendrogramme. The branches are labeled by the treatments of the corresponding plots. Visually, there is no relation between the treatments and vegetation groups. Somewhat clearer is the relation between the blocks and the vegetation groups, as shown in FIGURE 5.

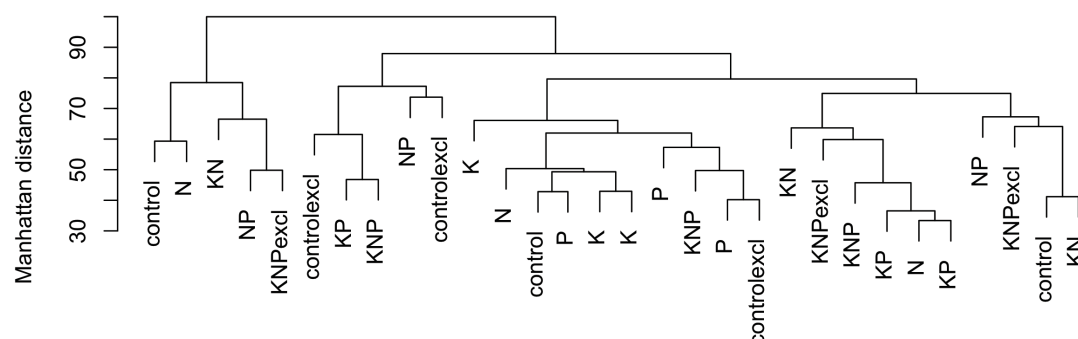


FIGURE 4. Dendrogramme showing the results of an agglomerative cluster analysis. Data basis was the cover of 92 species on each plot in late summer 2009. The labels of the branches show the treatments of the corresponding plots. N: nitrogen added, P: phosphorous added, K: potassium and other elements added (cf. methods), control: no fertilization, excl: exclosure of large herbivores.

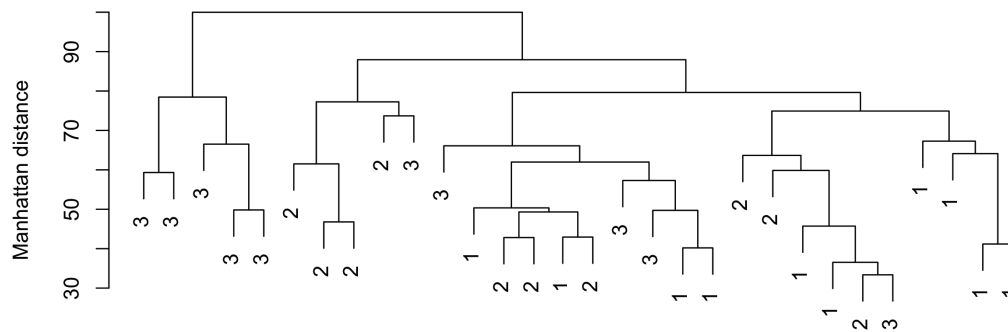


FIGURE 5. Dendrogramme showing the results of an agglomerative cluster analysis. Data basis was the cover of 92 species on each plot in late summer 2009. The labels of the branches show the block to which the corresponding plot belongs.

## 4. Discussion

H1: THERE IS A POSITIVE RELATIONSHIP BETWEEN DIVERSITY AND PRODUCTIVITY ON THE PLOTS.

Literature states the relationship between plant diversity and productivity as often being a humpbacked one (Grime 2002). Also, positive diversity-productivity relationships have been reported in experimental (e.g. Hector et al. 1999) as well as in observational approaches (Singh et al. 2005). The latter study deals with alpine grasslands and is thus quite comparable to the plots used here. Eventhough, the analyses presented here revealed no significant relation between vascular plant richness and productivity (measured as living aboveground biomass).

Mechanisms behind a positive diversity-productivity relationship include larger probability of having legumes in the community and (spatial and temporal) complementarity in resource usage of the different species (van Ruijven and Berendse, 2005). As can be seen in FIGURE 6, the number of leguminous species on each plot was not related to its aboveground biomass (also true for cover of leguminous species, data not shown).

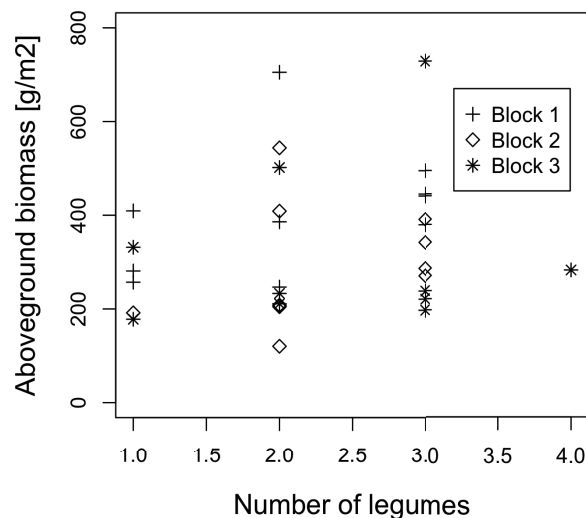


FIGURE 6. Aboveground biomass clipped in late summer 2008 and number of leguminous species on the plots.

Regarding complementarity of the species the data could be interpreted in a way, that the lowest plant species number on the plots (19) is already high enough to use all available resources (nutrient, light, water etc.) efficiently, so that there is no potential for further complementarity at higher diversities. Particularly, a visible effect of complementarity in terms of nutrient usage seems to be unlikely in the light of the independence of biomass and nutrient treatments (cf. results, Q1).

Still, using a wider range in plant diversity could lead to a relation between diversity and productivity as in Singh et al. (2005). If the number of species is below a certain threshold, this could result in unused resources and thus lower productivity. It might be worth testing this notion in future given the limited range of diversity in the plots used here (19-31). Additionally, the fact that exclusion of Block 3 and log-transformation of the biomass led to p-value smaller 0.2 can be seen as an encouragement towards follow-up studies in that respect.

**Q1: WHAT EFFECTS DOES FERTILIZATION WITH N, P AND K HAVE ON VASCULAR PLANT PRODUCTIVITY AND THE ABUNDANCES OF THE DIFFERENT PFTs?**

A comparable experiment to the one presented here was conducted in the Rocky Mountains by Bowman et al. (1993). They applied nitrogen, phosphorous and a combination of both to grasslands at 3510 m altitude, detecting N-limitation of the primary production on dry grasslands. Furthermore, abundance of graminoids increased as response to N-fertilization. On more productive wet grasslands, they also found evidence for competition between forbs and graminoids. In another experiment carried out in NW Caucasus, Russia at 2800 m a. s. l., Soudzilovskaia et al. (2005) found increased vascular plant biomass as reponse to N and N+P fertilization (although no overall increase in a community dominated by lichens).

The data presented here does not suggest any signal of fertilization on community aboveground biomass after one year. A first explanation could be, that the increased plant productivity remains undetected due to a

simultaneously increased herbivory. However, the aboveground biomass of plots prevented from herbivory was not higher (cf. TABLE 4). Data from 2009 suggest a trend towards a positive influence of the K<sup>+</sup> treatment, but this is not true for the differences 2009 – 2008. In case that trend would manifest in the future, this would be an interesting finding, as literature up to now rather focused on nitrogen and phosphorous, putting aside other nutrients.

Having no statistically significant results to date, the interpretation is, other environmental factors not controlled here are limiting community biomass. Potential factors include e.g. water, UV radiation and temperature. To analyse this in further depth, additional experiments are needed.

Given the high inter annual and spatial variability in aboveground vascular plant biomass, it might also well be that the following years (over which the treatments will be continued) reveal some kind of a relation. Compared to the literature reporting treatments over two (Bowman et al. 1993) and four (Soudzilovskaia et al. 2005) years time, one year period of treatment application is relatively short.

Regarding the abundance of the different PFTs, a first hypothesis could have been to expect a relation between legumes and N-fertilization, since the capability to fix nitrogen gives legumes a competitive advantage, which is no more present when fertilizing nitrogen (e.g. Silvertown et al. 2006). However, no effect of nitrogen is obvious here. This might be due to the small (1-2%) and highly variable abundance of legumes on the plots. In the consequence, no indirect competitive effect of legumes on other PFTs is to be expected.

The plots are naturally dominated by graminoids and forbs. Both show some response to nitrogen fertilization. While the 2009 data only showed 'trends', this was supported by the consistent responses of the per-plot differences 2009-2008. Adding nitrogen increased the abundance of forbs, but there is evidence that this only happens when adding also potassium and other elements. At the same time, adding nitrogen decreased the abundance of graminoids, in contrary to the findings by Bowman et al (1993). Taken together, these findings suggest that forbs are, under the given environmental conditions, able to make use of additional nitrogen if additional elements are added. Instead of an increased community biomass, this leads to an increased abundance of forbs and a decreased abundance of graminoids, which are apparently less competitive. Other resources beside nutrients seem to limit community growth overall, as stated above.

Regarding woody species, the analysis of the on-plot differences 2009 – 2008 revealed a significant negative influence of phosphorous fertilization of woody species abundance. Even though there is no positive relation between phosphorous and any other PFT, this could still be a result of competition, given the already low abundance of woody species. In general, the presented data is only of limited use to analyse woody species at this scale, since they only occurred in very few plots (8 out of 30).

**Q2: DOES THE EXCLOSURE OF LARGE HERBIVORES EFFECT VASCULAR PLANT BIOMASS AND THE ABUNDANCES OF THE DIFFERENT PFTs?**

Exclosure led to no significant effect, neither in community biomass nor in abundances of the PFTs. The mean total biomass on unfenced plots was even higher ( $334 \text{ g}\cdot\text{m}^{-2}$ ) than on fenced plots ( $273 \text{ g}\cdot\text{m}^{-2}$ ). This could even mean an negative effect of the fences, maybe by reducing the available light.

This leads to the tentative conclusion, that the herbivore pressure on the plots is already quite low, respectively the plants are well adapted to it. With a low pressure on overall biomass, also no potential favoring of particular PFTs by the herbivores could manifest.

**H2: THE TREATMENTS LEAD TO CHANGES IN THE VEGETATION PATTERN.**

Nutrient availability is a crucial factor structuring communities at least over the long time (Silvertown et al. 2006). Spiegelberger et al (2006) report a revisit of an experiment conducted on subalpine grassland in the 1930s. NPK fertilizer was applied over three years and led to a significant shift in species composition of the vegetation. Even when they revisited the plots about 70 years later, the signal of the NPK fertilizer was still significant on the 10%-level ( $p = 0.083$ ).

This supports the assumption that one growth period was simply a too short period for the fertilizer application to manifest in the vegetation. The vegetation seems to be somewhat structured along the blocks, the fertilizer effect needs to be stronger than this structure in order to be visible in the vegetation. Given the literature findings mentioned, it can be assumed that the relation between treatments and vegetation structure will become somewhat clearer in the consequent years.

## **5. Conclusions**

The work at hand presented a preliminary analysis of the Swiss site of NutNet initiative. The results hold implications for the diversity-productivity debate, the role of nutrient limitations on alpine grasslands and the effect of large herbivores in structuring and limiting alpine vegetation. Even though the treatments have only been applied over the short period of one alpine summer, some quite interesting results were revealed.

In terms of a diversity-productivity-relationship, no significant relation was found, still the results are somewhat encouraging to continue work using a broader range of species richness.

The influence of fertilization with especially nitrogen was particularly interesting. Although community biomass remained constant, the competitive relationship between forbs and graminoids changed. This happened in a way that forbs could benefit more from fertilization, also they needed the combination N&K+ to make use of the fertilization. This result is in contrast to other studies and shows that the influence of nitrogen on forbs-graminoids-competition is



context-dependent. Also there was some significant signal of fertilization on the level of PFTs, this was not true on the species level. Given that almost all alpine plants are perennial, it is quite obvious, that this will take some time. It will be interesting to see over what period fertilization has to be applied to leave a footprint in vegetation at species level. Literature already showed, that this footprint can be profound and long-lasting.

The least encouraging results were related to herbivore exclosure, even though one might have expected results after one year, at least on community biomass level. Further methodological clarification concerning herbivory pressure and effectiveness or even negative influences on vegetation of the fences might be advisable.

By integrating herbivory and nutrient treatments in one experimental design on a global scale, the NutNet initiative is about to make important contributions to the understanding of the nutrient cycling and the trophic interactions in grasslands. One year after the first treatments, encouraging results can already be found on the scale of a single site. The following years will help clarifying some open question and lead to insights about effects of herbivory and nutrients on grasslands in the mid- to long-term.

## 6. References

- Bowman, W. D., et al. 1993. Constraints of Nutrient Availability on Primary Production in Two Alpine Tundra Communities. *Ecology* **74**:2085-2097.
- Braun-Blanquet, J. 1964. *Plant sociology: the study of plant communities*. Hafner, London.
- Grime, J.P., 2002. *Plant strategies, vegetation processes and ecosystem properties*. Wiley, Chichester.
- Hector, A., et al. 1999. Plant Diversity and Productivity Experiments in European Grasslands. *Science* **286**:1123-1127.
- Mosteller, F., and J. W. Tukey. 1977. *Data Analysis and Regression: A Second Course in Statistics*. In: *Behavioral Science: Quantitative Methods*. Addison-Wesley, Reading, MA.
- Shapiro, S. S., and M. B. Wilk 1965. An Analysis of Variance Test for Normality (Complete Sample). *Biometrika* **52**:591-611.
- Silvertown, J., et al. 2006. The Park Grass Experiment 1856-2006: its contribution to ecology. *Journal of Ecology* **94**:801-814.
- Singh, S. P., et al. 2005. Species diversity contributes to productivity – Evidence from natural grassland communities of the Himalaya. *Current Science* **89**:548-552.
- Soudzilovskaia, N. A., et al. 2005. Biomass production, N:P ratio and nutrient limitation in a Caucasian alpine tundra plant community. *Journal of Vegetation Science* **16**:399-406.
- Spiegelberger, T., et al. 2006. Long-term effects of short-term perturbation in a subalpine grassland. *Ecology* **87**:1939-1944.
- Stevens, C. J., et al. 2004. Impact of Nitrogen Deposition on the Species Richness of Grasslands. *Science* **303**:1876-1879.
- Van Ruijven, J., and F. Berendse 2005. Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. *PNAS* **102**:695-700.