Low habitat effects on the diet quality-composition relationship in Corsican mouflons.

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ABSTRACT

Population management plans are usually based on landscape actions. Diet quality and composition of ungulates affect their fitness, while composition is influenced by landscape. In this work we described the relationship between diet quality and composition of Corsican mouflon (*Ovis gmelini musimon var. Corsicana*). We also evaluated the habitat variations in the link between diet quality and composition of this mountain ungulate. A total of 103 faecal samples from Cinto, Corsica were collected in autumn 2016. Diet quality was assessed by near infrared reflectance spectroscopy (NIRS) whereas diet composition by cuticle microhistological analysis of faecal samples. Diet composition of mouflons was particularly rich in woody plants. Faecal nitrogen, a proxy for diet quality, was negatively related with Graminoids while positively with Herbaceous plants. A positive but non-significant relationship between diet quality and Woody plants was observed. A high proportion of graminoids in their autumn diets will result in a poor diet quality. A lower quality diet, however, is driven by open habitats dominated by graminoid-like species. In a woody habitat, consumption of woody plants seemed to compensate for the low nutritional value of graminoids-rich diets. Hence, a reduction in the proportion of habitats rich in woody plants will result in a decrease in the quality of mouflon diets during autumn.

Keywords: Faecal nitrogen, *Ovis musimon*, microhistology, graminoids, landscape.

INTRODUCTION

Understanding temporal and spatial variations in feeding habits of wild ungulates is crucial for implementing population management plans based on landscape modifications (Marchand et al. 2014). In fact, ungulates are primary consumers, notably regulated by bottom-up mechanisms driven by intra and inter-specific competition for food resources (Hopcraft et al. 2010; Pierce et al. 2012).

But, most of the works on this sense has been focused on seasonal patterns (Cransac et al. 1997; Owen-smith 1994; Parker et al. 2009), and few efforts have been made to understand feeding variations at habitat or landscape scale (but see Abbas et al. 2011; Seagle and Mcnaughton 1992; Zweifel-schielly et al. 2009). In fact, such spatial variations in feeding habits are considered one of the drivers for diet composition (Christianson and Creel 2007; Gebert and Verheyden 2001) in ungulates. For instance, Christianson and Creel (2007) found that winter graminoid consumption by elk was higher in open meadows and grasslands than during the rest of the year.

Northern ungulates spend most part of their time feeding to increase their body reserves in anticipation of food shortages during winter (Parker et al. 2009). Hence, survival and reproduction rates of ungulates are mainly driven by their nutritional status (Parker et al. 2009).

In mixed feeder ungulates, both diet composition and quality mainly rely on plant availability and hence on landscape characteristics (Codron et al. 2007; Martinez 2010). In fact, up to 33% of diet variations of red deer (*Cervus elaphus*), a mixed feeder, relies on the habitat characteristics (Gebert and Verheyden, 2001). Though the clear importance of habitats to understand the feeding preferences of ungulates, few efforts have been made to explore the relationships between diet quality and composition at landscape scale in most of ungulate species.

The European mouflon is a medium sized mixed feeder inhabiting a broad range of habitats ranging from the Alpine ecosystem of Bauges, France to the dry and semiarid landscapes of Spain (Marchand et al 2013). Mouflon is also common in islands such as Hawaii, Kergelen, Corsica, Ciprus and Sardinia (Marchand et al 2013).

In general, mouflon behave as a mixed feeder. Mouflons' diet are mainly based on graminoids and herbaceous (Cransac et al. 1997; Berrocal and Molera 1985; Garcia-Gonzalez and Cuartas 1989)with some proportion of woody plants. Curiously, this proportion of woody plants can be extremely high in Corsica, reaching the 74% of food intake (Pfeffer 1967).

Because its great dietary plasticity (Marchand et al 2013) and the huge variability of habitats and vegetal communities in Corsica, the Corsican mouflon (*Ovis gmelini musimon var. Corsicana* Cugnasse 1994) is an excellent model for exploring landscape variations in diet quality and composition in ungulates. Although there is some preliminary information about the feeding habits of Corsican mouflon (see Pfeffer 1967), little information exist about diet quality variations in this caprinae species (Bourgoin et al. 2008). No works, however, have explored neither diet quality of Corsican mouflon nor the relationships between diet quality and composition at landscape scales (but see Redjadj et al. 2014 for a seasonal approach on quality and landscape).

In addition, Mediterranean ecosystems (Resco De Dios et al. 2007), and in particular Mediterranean islands (Gritti et al. 2006), are especially vulnerable to landscape changes due to wildfires (Mouillot et al. 2005). Hence understanding the role of landscape on the feeding habits of Corsican mouflons will be useful to improve landscape management plants oriented to preserve this emblematic sheep.

In this work we used 103 mouflon faecal samples from the Cinto area, Corsica, with a threefold purpose: (I) to describe diet quality and composition of mouflons from this population; (II)to explore the relationships between diet composition and diet quality and (III) to evaluate habitat and quality-composition relationships in this mountain ungulate.

MATERIAL AND METHODS

Study area

Corsica is a 8680 km^2 French island, located at 42°09′00″ North and 9°05′00″ East in the Mediterranean sea, at the south-east of France and at the west of the Italican peninsula. A mountain range crosses the island in NW-SE direction, being Mount Cinto the highest peak (2706 m.a.s.l). The samples were obtained all from a ~200 km^2 area in the northern part of the Parc Naturel Regional de Corse, corresponding to the Cinto mouflon population.

The island shows a typical Mediterranean climate with dry summers. However, significant variations in temperature and precipitation from shorelines to high peaks favours the existence of different microclimates ranging from the humid Mediterranean climate to the alpine climate (Gamisans 1991). In the sampling area, annual average temperature is 8.9ºC and 27ºC and 1ºC the maximum and minimum annual temperatures respectively. November the rainiest month in Corsica (Asco ski station meteorological data at 1423 m.a.s.l).

Five vegetation communities dominate the Corsican landscapes (Rome and Giorgetti 2007). From the sea level to 600 m.a.s.l., landscape is dominated by cork oak (*Quercus suber)*, evergreen oak (*Quercus ilex)*, stone pine *(Pinus pinea)* and Aleppo pine *(Pinus halepensis*). From 400 to 1200 m.a.s.l., landscapes are rich in maritime pine (*Pinus pinaster)*, chestnut (*Castanea*), Italian alder (*Alnus cordata*), European hephornbeam (*Ostrya carpinifolia*), sessile oak (*Quercus petraea*), pubescent oak (*Quercus pubescens*) and evergreen oak (*Quercus ilex).* From 1000 to 1800 m.a.s.l., vegetation is defined by firs (*Abies*), European beech (*Fagus sylvatica*) and black pine (*Pinus nigra).* At higher altitudes (1800-2100 m.a.s.l.) genista (*Cytisus, Genista)* can be found as well as thimes (*Thymus*), Corsican sweet alder brush (*Alnus viridis*) and junipers (*Juniperus*) among others. Finally, at the highest strate (>2100 m.a.s.l.) *Poa violacea* meadows dominate the scenery.

Sampling procedure

From 03-Oct-2016 to 13-Dec-2016, 103 faecal samples were collected in the Cinto area (646 to 1985 m.a.s.l.) by a ranger from the Office National de la Chasse (A. Baconnier). Most of faecal samples (86%) were fresh while others (14%) were collected on the ground. Faecal samples were collected in plastic labelled bags and frozen until analysis. After, frozen samples were thawed and dried in an oven at 60ºC for 24h and grinded with a 1 mm pitch laboratory mill (Cyclotec 1093, FOSS Tecator, Höganäs, Sweden).

Diet quality assessment

Faecal nitrogen (FN) is a widely used and useful indicator to assess diet quality (Clauss et al. 2013; Kamler and Homolka 2005; Monteith et al. 2014; Ramanzin et al. 2017; Redjadj et al. 2014; Villamuelas et al. 2016, 2017). Otherwise, neutral detergent fibres (FND = cellulose + hemicellulose + lignin), acid detergent fibres (FAD = cellulose + lignin) and acid detergent lignin (LAD = lignin) are useful to complement faecal nitrogen as normally both proxies follow contrary patterns (Gaidet and Lecomte 2013; Ramanzin et al. 2017; Steuer et al. 2014)

We employed the non-destructive Near Infrared Spectrophotometry (NIRS) to asses nitrogen and fibre contents. Samples were deposited in quartz based circular cups of 35 mm diameter and analysed in the NIRSystems 5000 spectrometer (FOSS, Hillerød, Denmark), using a wavelength from 1100 to 2500 nm. We obtained data in 2nm intervals as log 1 / R, being R the reflectance. Each sample was scanned in duplicate by performing a 180 ° rotation of the cup. If the sample was so small as not to cover the entire quartz glass base, a base was added to the cup, reducing the surface to half, in such a way that two readings were made, each with its duplicate.

Faecal nitrogen data was extracted using the multi-specie equation (Villamuelas et al. 2017). Afterwards, FN was corrected with neutral detergent fibres (FND) to supress the potential bias due to possible ingestion or contamination with soil (Ramanzin et al. 2017).

For the fibre content we also analysed 10 samples in the laboratory following the Van Soest system (Van Soest et al. 1991) using an Ankom 220 Fibre Analyser (ANKOM Technology, USA). Then, we used the values obtained from the 10 samples in the laboratory to make an adjustment of the equation used to extract our data and we extract it again corrected.

Diet composition

The cuticle microhistological analysis technique is useful to estimate botanical composition of diets using faecal samples (Bartolome et al 1995, 1998). This technique has extensively been used to determine diets of other mountain ungulates (Villamuelas et al. 2016; Ihl et al 2001; Chapuis et al 2001). Between 2 to 3 gr from each sample was placed in a test tube and mixed with 3 ml of HNO_3. Then, the tubes were boiled for 1 minute at 80 ºC and the content of each tube was drained in different bakers and diluted with 200 ml of distilled water. This suspension was then strained using two sieves 1 mm and 0.125 respectively. The remaining portions were spread on glass slides with glycerine and fixed with cover-slips with DPX microhistological varnish. Two slides per sample were prepared. Later, we examined the slides under an optical microscope at 10x and 40x magnification following five transects for each slide. Using an epidermis collection, we recorded leaf fragments at genus or specie level when possible. Results have been expressed as the proportion of given plant species over the total of fragment found.

Habitat data

For each sample we centered on its spatial coordinates a buffer of a 550 meters radius. Radius size was determined based average weekly home range size of mouflon in Corsica obtained from 18 GPS collared animals (13 males and 5 females). Then, all the values of proportion of habitats related to the pixels included in the buffer range were extracted: proportion of grassland, moorland, caducifolious forest, riparian forest, coniferous forest, *Alnus* forests, maquis shrublands, habited zone, rocks, clear forest and superficial water.

Statistical Analysis

For statistical purposes we used plant groups (Graminoids, Herbaceous and Woody) instead of plant species. Not classified plants (group Others) was not taken into account because of its low relevance (2.3% of plants).

For quality-composition relationships, we performed a Principal Components Analysis (PCA) to assess which microhistological categories were more related to fibre or nitrogen. Then, we used linear models to test the relationship between each group of plants and diet quality (FN) of mouflons. Finally, the influence of landscape on the relationship between the proportion of plants in the mouflon diets and its FN was also assessed by linear models.

All statistical analyses were performed using the statistical software R (version 3.5.1, The R Developement Core Team 2018). Multivariate analysis was conducted using the FactoMineR package 1.41 version (Le et al. 2008).

RESULTS

Diet composition

Most of fragments were difficult to classify. Over the 17105 fragments, only 5381 (31,46%) were classified up to species level, while 11724 (68,54%) were sorted into “Other graminoids”, “Other herbaceous”, “Other woody” and “Others”. The principal group of plants eaten by Corsican mouflons was Other woody, followed by Other graminoids. The identified species with higher importance were *Festuca ovina* and *Quercus spp.* (Tab. 1).

Regarding the general groups, the more consumed group was Woody (60.3 %), followed by Graminoids (26.7 %), Herbaceous (10.7 %) and finally Others (2.3 %).

Diet quality

The overall mean for corrected FN was 4.239 ± 0.897 and 2.125 ± 0.288 for non-corrected FN. The NDF mean content was 51.050 ± 5.150, whereas 35.075 ± 4.433 for ADF. Finally, 16.142 ± 3.241 was the mean content for ADL.

Diet composition and quality relationships

The first PCA dimension was mainly described by diet quality components whereas the second by microhistological categories (Fig. 1). First two dimensions explained 66.8 % of the observed variability in the diet quality and composition parameters of Corsican mouflons. ADF and NDF contributed positively and strongly to the variability of the Dimension 1 (38.3 % of variance). ADL also contributed in a positive way but in a lower degree. On the other hand, FN had a strong and negative contribution to the variability of Dimension 1. In Dimension 2 (28.5% of variance) there was a strong contribution of the plant groups Woody (positively) and Graminoids (negatively). FN and ADL also contributed positively but to a lesser extent to the Dimension 1 and NDF contributes a slightly to Dimension 2. Herbaceous and Others did not contribute significantly to either the Dimension 1 nor the Dimension 2.

Through linear models, we detected a negative and significant correlation between diet quality and Graminoids (t value = -2.174 ; P= 0.0321, Fig. 2) but positive with Herbaceous contents (t value= 3.14; P= 0.0186, Fig. 3). On the other hand, we found a non-significant positive relationship between FN and the proportion of Woody plants in our mouflons' diet. (t value = 1.440; P= 0.153, Fig. 4).

Diet quality-composition relationship through habitats

Through linear models we assessed FN through an interaction between habitat and Graminoids, Herbaceous and Woody (Fig. 5, 6 and 7 respectively). For the FN-Graminoids relationship, we failed to detect a significant interaction with coniferous forest (t value= -0.031; P= 0.975), grassland (t value= -0.141; P= 0.888), caducifolious forest (t value= -0.019; P= 0.985), or *Alnus* forest (t value= 0.578; P= 0.565). On the other hand, we found a significant interaction between Graminoids and FC in maquis shrubland (t value= 2.102; P= 0.038) and moorland (t value= -2.498; P=0.014). We did not find any significant interaction for FN-Herbaceous: coniferous (t value= 0.067; P= 0.946), maquis shrubland (t value= 0.296; P= 0.768), grassland (t value= -0.029; P= 0.977), caducifolious forest (t value= 0.779; P= 0.438), moorland (t value= 1.397; P= 0.163) and *Alnus* forests (t value=-1.697 ; P= 0.093). Along the same lines, no significant interactions between Woody plants and FN were observed in coniferous (t value= 0.104; P= 0.918), maquis shrubland (t value= -1.468; P= 0.145), grassland (t value= -0.113; P= 0.910), caducifolious forest (t value= -0.079; P= 0.937), moorland (t value= 1.131; P= 0.261) and *Alnus* forest (t value= 0.509; P= 0.612). A post-hoc Spearman correlation found a negative correlation between maquis, shrublands and moorlands in the study area.

DISCUSSION

With regard to the first objective of this thesis, in line to a previous work, diet composition of Corsican mouflons was mostly based on woody plants (61.24%). This proportion of woods can be even higher (75%) as observed in the Corsican mouflon south population (Pfeffer 1967). Woody intake in Corsica is quite high compared to other populations. In Bauges, France, for example (Babad 1997) mouflons consume 26% of woody plants whereas 7.8 % in south Spain (Garcia-Gonzalez and Cuartas 1989). This observation, emphasizes the great feeding plasticity of this ungulate (Marchand et al 2013) and its ability for colonising a wide range of habitat types (Cransac et al 1997).

Regarding the relationship between the diet composition and diet quality, our results suggest that diets rich in graminoids are nutritionally poor.

The fact that samples were collected in autumn will explain such low contribution of graminoids in terms of diet quality (Aublet et al. 2009).

Regarding the habitat effects on the diet quality of Corsican mouflons, a larger proportion of maquis shrublands appear to improve the Graminoids-FN ratio. The opposite was observed in samples collected in moorland, where a decrease in the quality of Graminoids was observed in moorland dominated areas. The negative Spearman's correlation coefficient confirmed that areas rich in maquis shrubland are poor in moorlands. Mixed grazers diet can include high proportion of graminoids but also high proportion of woody species (Marchand et al 2013) depending on the available vegetation. In autumn, when graminoids are low in quality, moorlands would constitute a low-quality habitat. On the other hand, shrublands in the Mediterranean basis in autumn offers high quality forage for ruminants (Kökten et al. 2012). In our study, even the positive relationship found between Woody and quality was not significative, in a woody habitat, woody consumption appear to compensate for the low nutritional value of graminoids-rich diets.

The Corsican mouflons have an extremely rich woody diet in comparison with their European counterparts. A high proportion of graminoids in their autumn diets will result in a poor diet quality.

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TABLES

Table 1 Diet composition of mouflons in Cinto, north Corsica based on cuticle microhistological analyses of faeces. Values represent percentage of observed fragments.

Diet composition of mouflons in Cinto, north Corsica					
	Plant	Mean (%)	SE	Min (%)	Max (%)
Woody	*Quercus spp.*	6.5	1.3	0.0	75.1
	Rubus spp.	4.5	1.0	0.0	66.7
	Erica spp.	2.6	0.4	0.0	20.0
	Pinus spp.	1.1	0.3	0.0	22.9
	Juniperus spp.	0.1	0.0	0.0	1.1
	Other woody	45.5	1.9	6.0	85.2
	Total	60.3	22.3	12.3	22.4
Graminoids	*Brachipodium spp.*	4.0	0.5	0.0	30.5
	Festuca ovina	7.0	1.1	0.0	62.6
	Carex spp.	0.1	0.1	0.0	5.5
	Other graminoids	15.6	1.2	0.0	49.3
	Total	26.7	20.7	0.0	86.5
Forbs	*Cystus spp.*	0.3	0.1	0.0	9.5
	Pteridium aquilinum	0.2	0.1	0.0	6.0
	Other herbaceous	10.2	1.0	0.0	63.8
	Total	10.7	10.4	0.0	63.8
Others	**Total**	2.3	4.8	0.0	22.4

FIGURE LEGENDS

Figure 1 PCA distribution for the quantitative variables of mouflon diets: quality components (NDF, ADF, FN) and composition (Graminoids, Herbaceous, Woody and Others). Colours show contributions of each variable to the construction of the PCA.

Figure 2 Conditional plot representing variation of corrected faecal nitrogen (FN) percentage related to Graminoids abundance in faeces of mouflon in Cinto, north Corsica.

Figure 3 Conditional plot representing variation of corrected faecal nitrogen (FN) percentage related to Herbaceous abundance in faeces of mouflon in Cinto, north Corsica.

Figure 4 Conditional plot representing variation of corrected faecal nitrogen (FN) percentage related to Woody species abundance in faeces of mouflon in Cinto, north Corsica.

Figure 5 Conditional plots representing the relationship between Graminoids and corrected faecal nitrogen (FN) through different proportions of habitat (coniferous forest, maquis shrubland, grassland, caducifolius forest, moorland and alnus forest).

Figure 6 Conditional plots representing the relationship between Herbaceous and corrected faecal nitrogen (FN) through different proportions of habitat (coniferous forest, maquis shrubland, grassland, caducifolius forest, moorland and alnus forest).

Figure 7 Conditional plots representing the relationship between Woody and corrected faecal nitrogen (FN) through different proportions of habitat (coniferous forest, maquis shrubland, grassland, caducifolius forest, moorland and alnus forest).

FIGURES

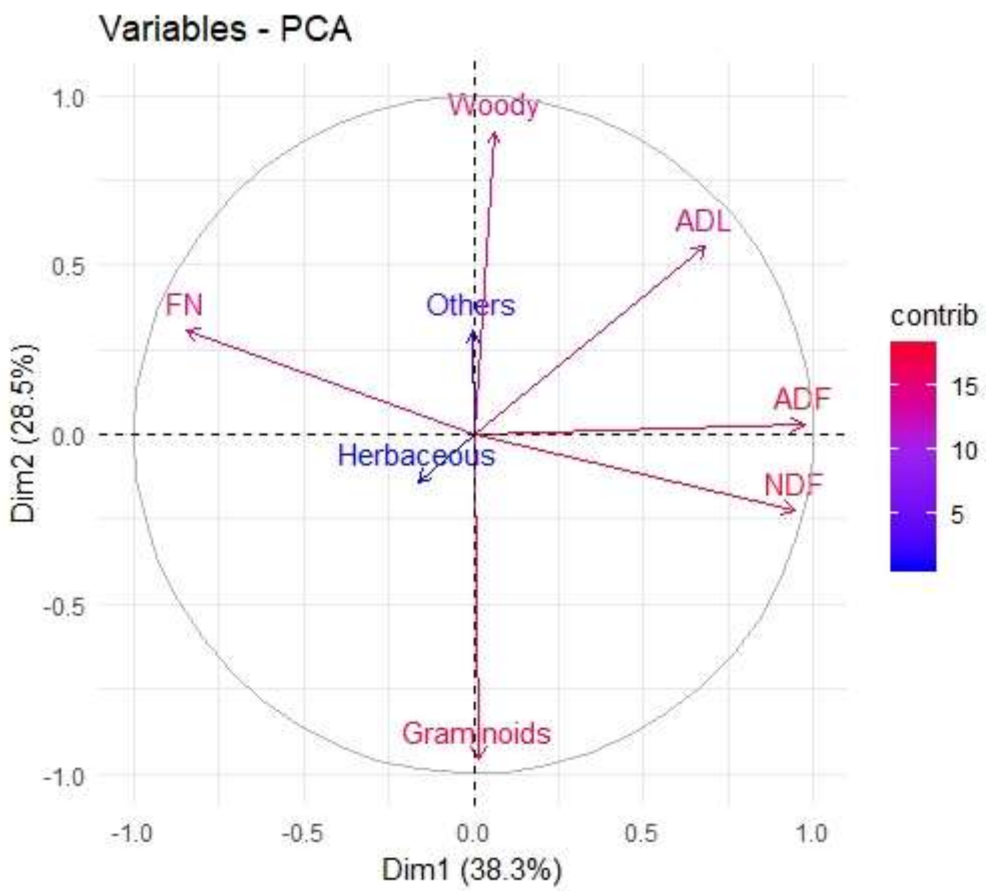


Figure 1

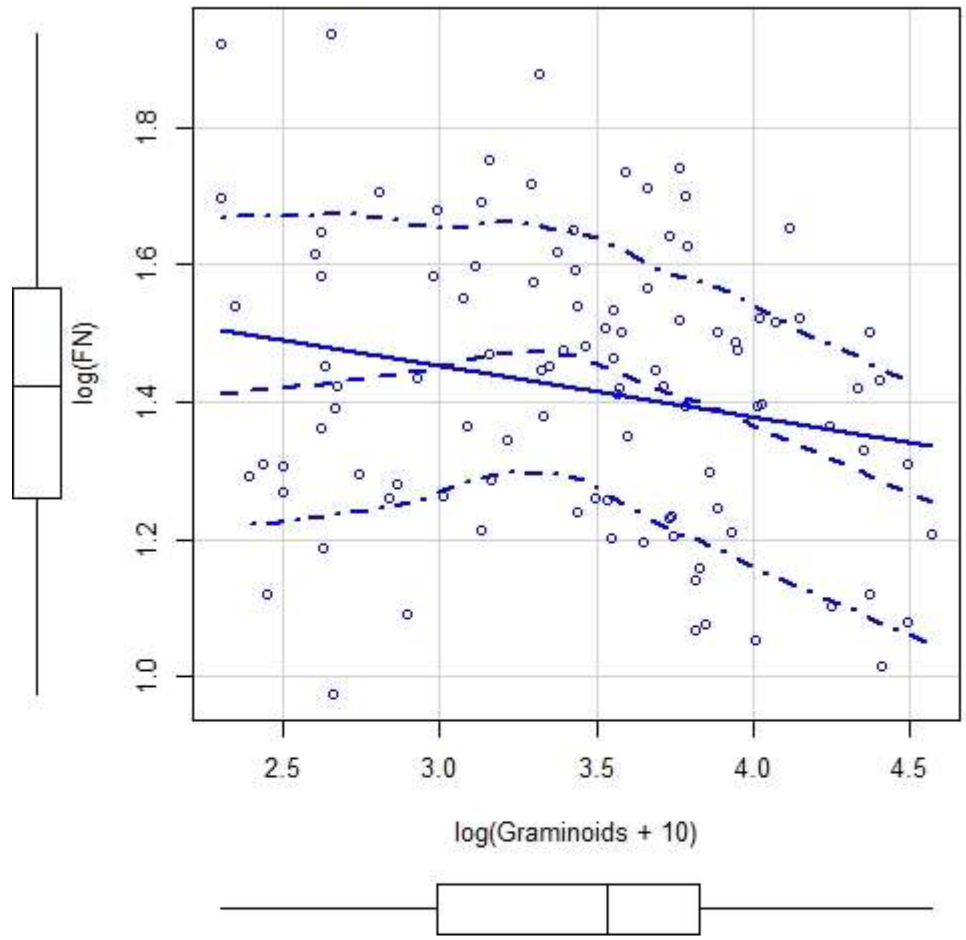
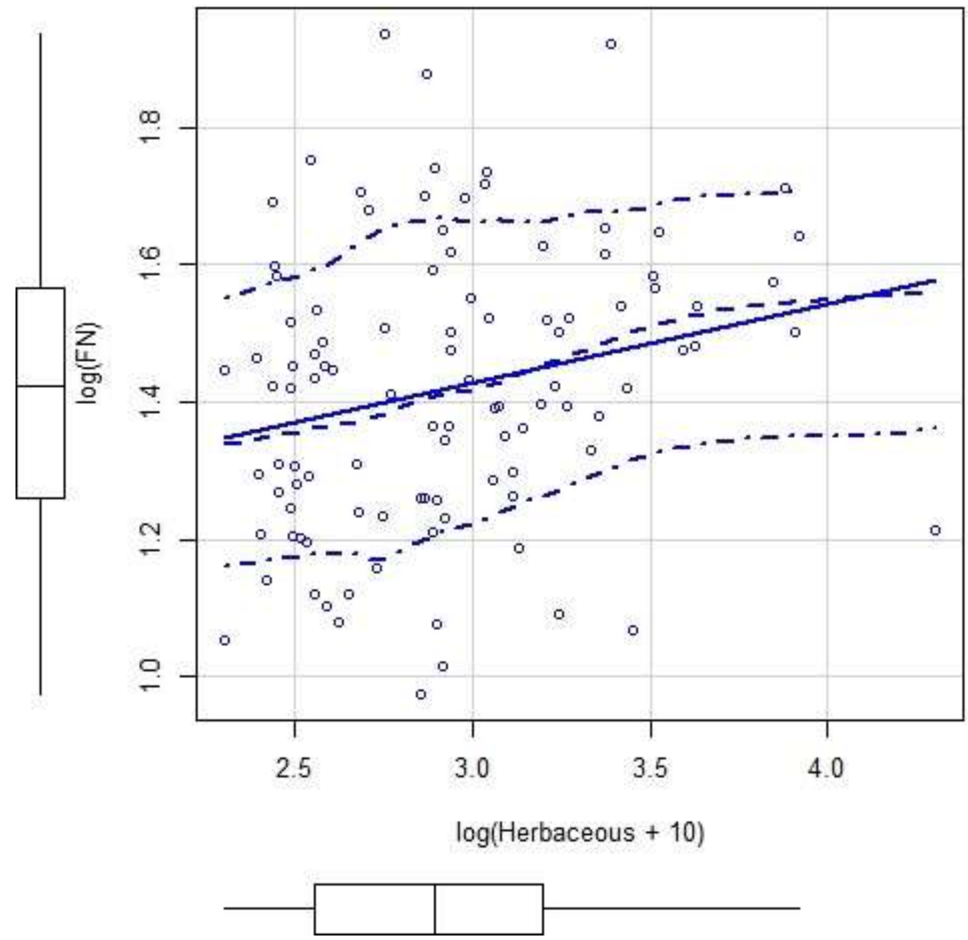


Figure 2



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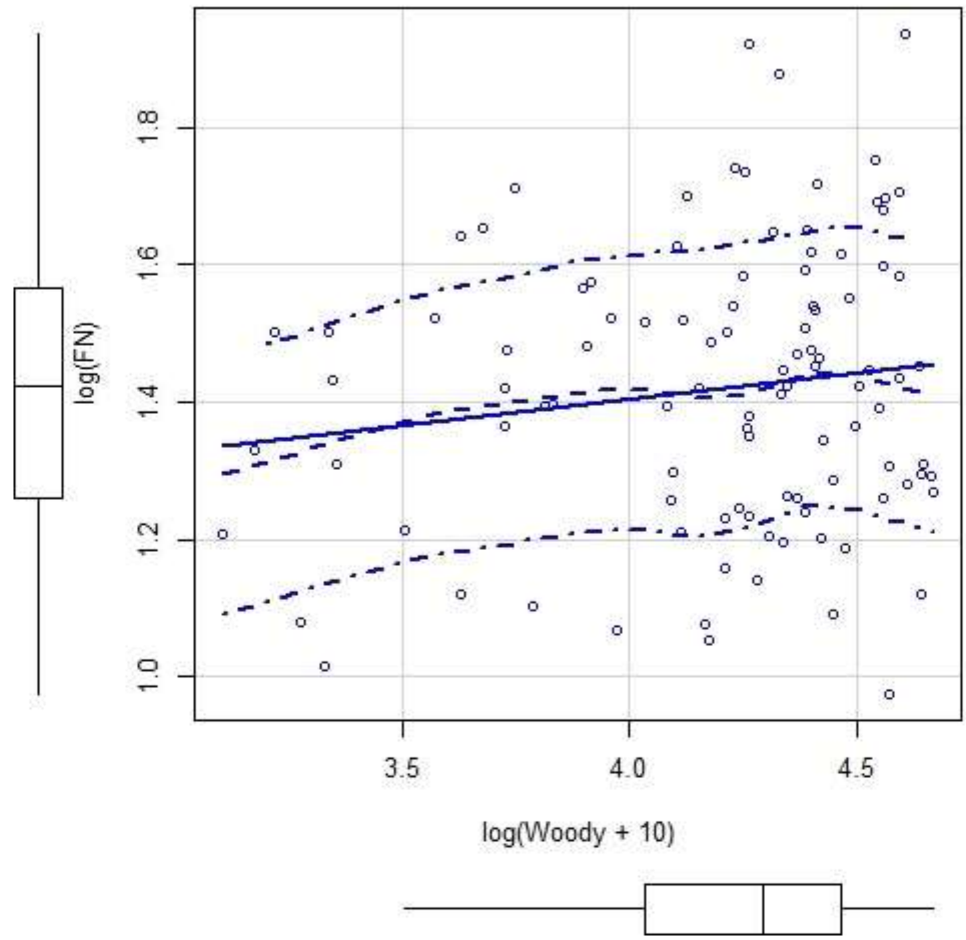

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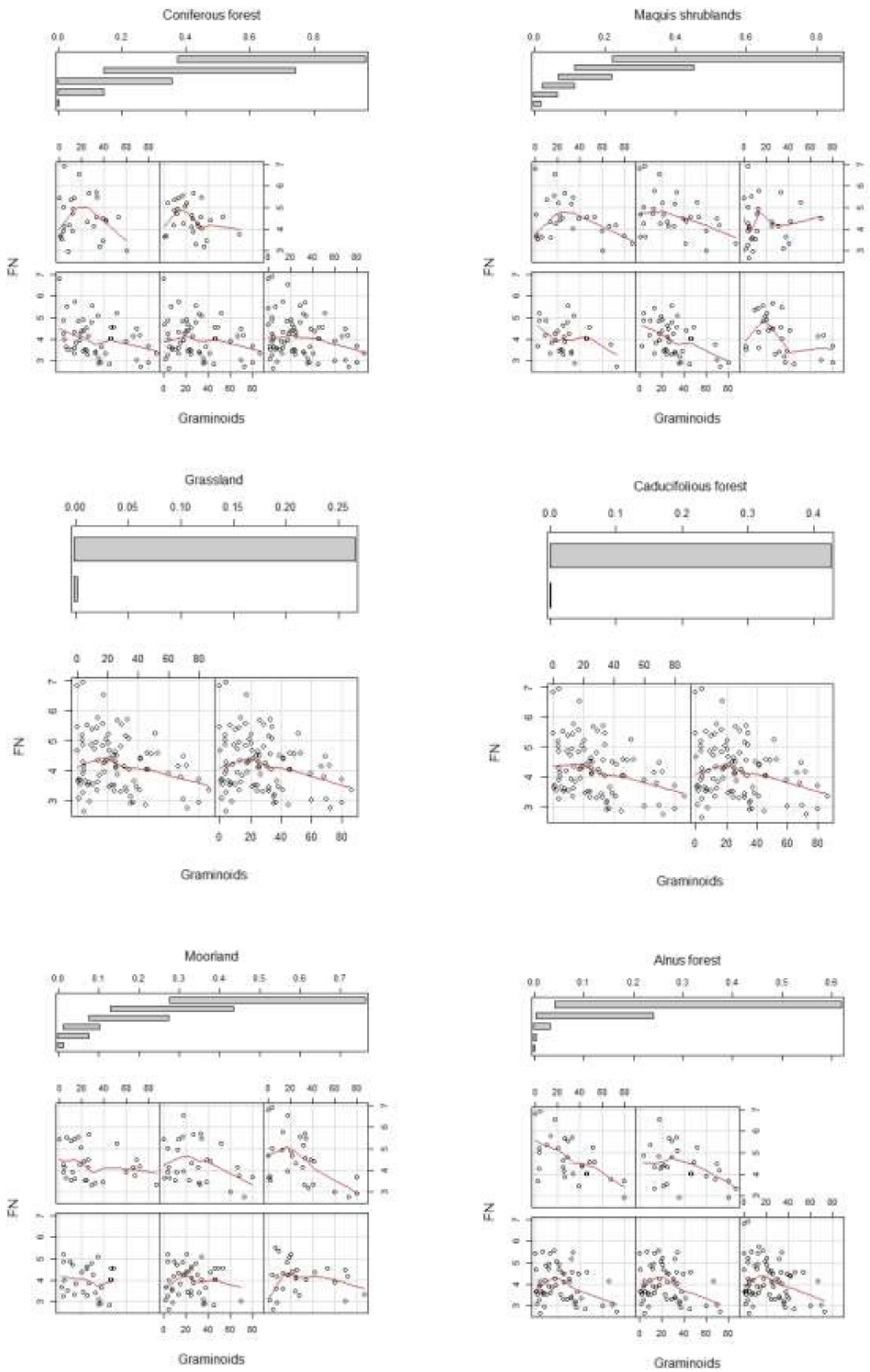

Coniferous forest
Maquis shrublands
Grassland
Caducifolious forest
Moorland
Alnus forest
FN
Graminoids

Figure 5

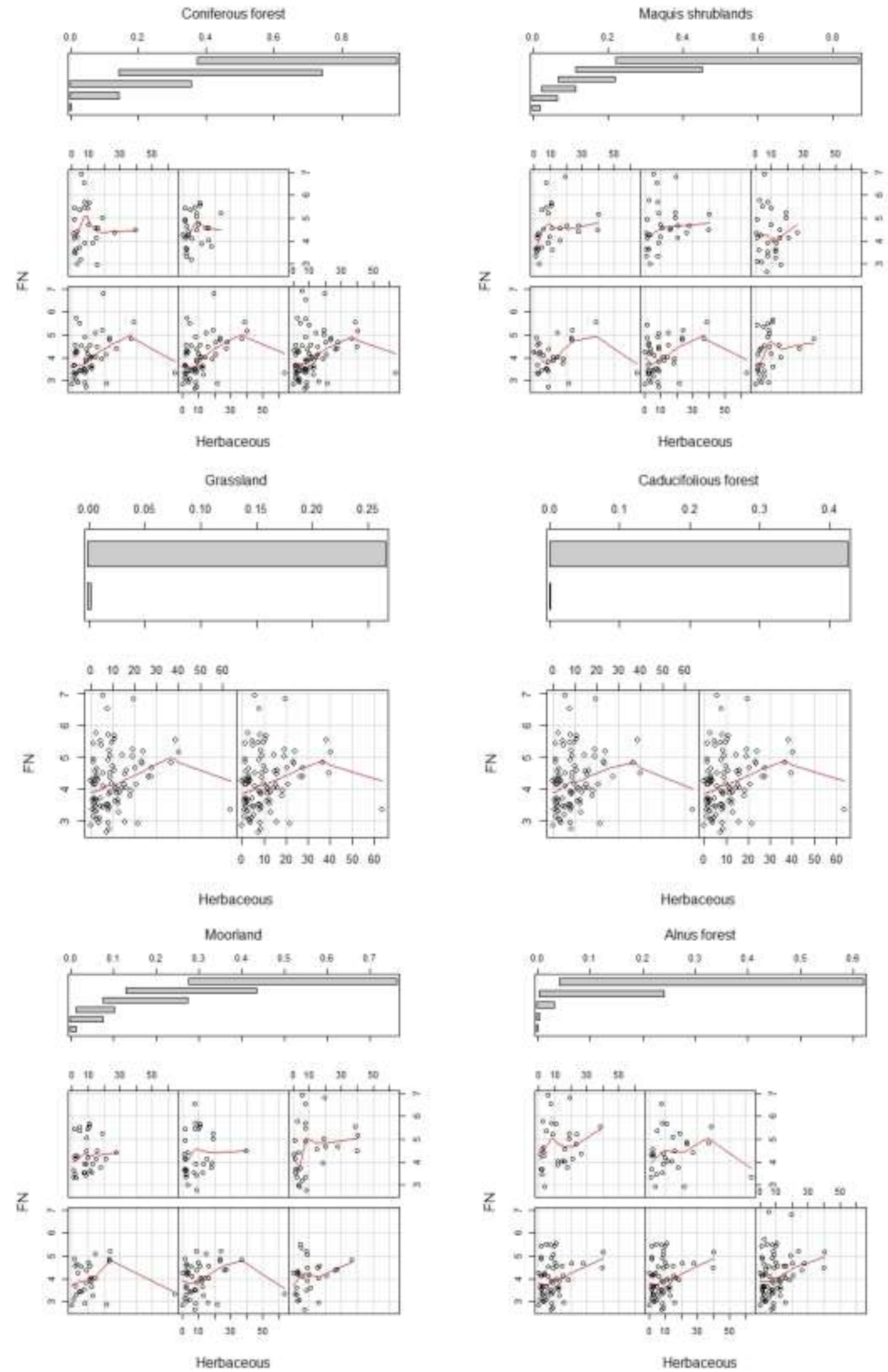
Coniferous forest
Maquis shrublands
FN
Herbaceous
Grassland
Caducifolious forest
FN
Herbaceous
Moorland
Alnus forest
FN
Herbaceous

Figure 6

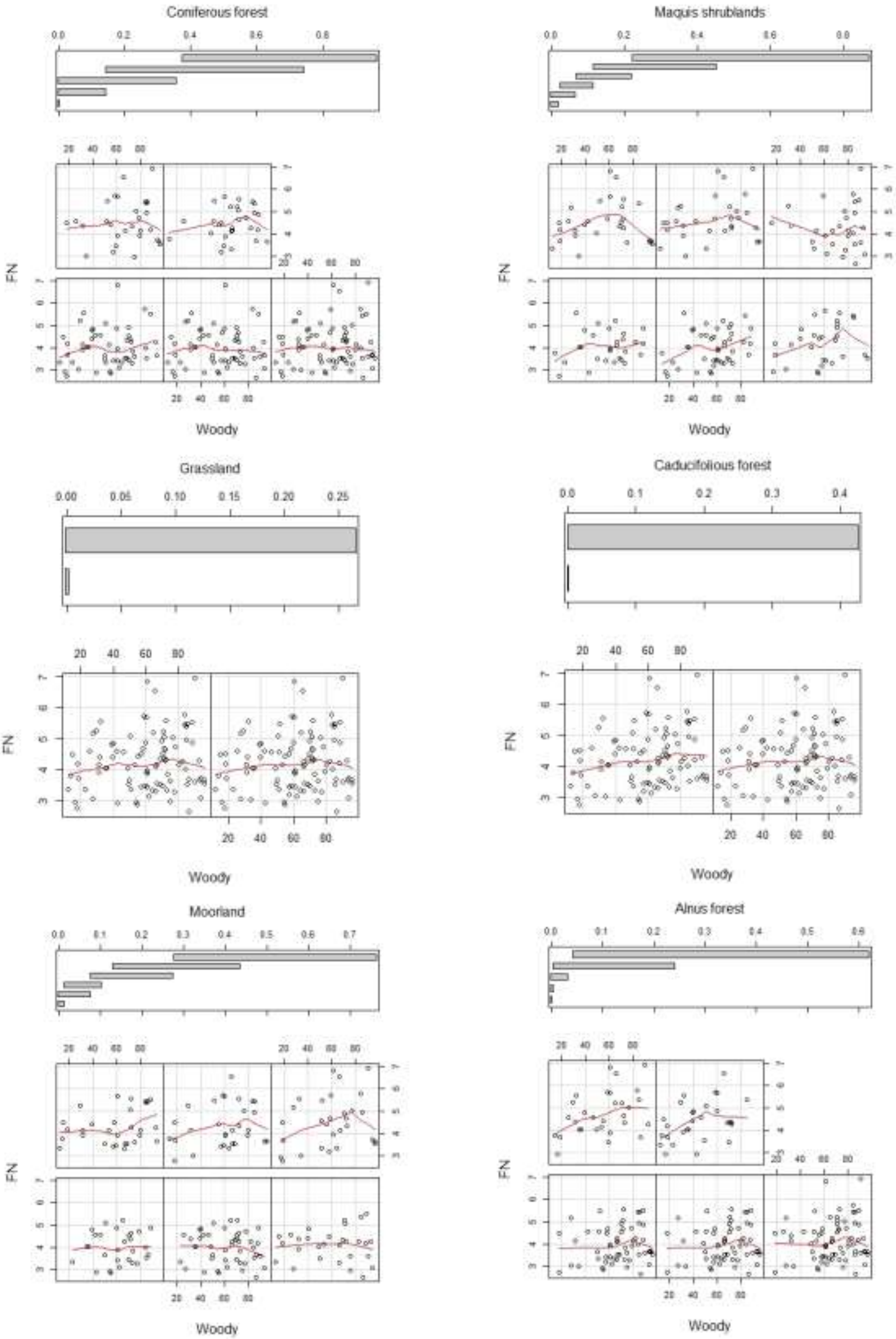
Coniferous forest
FN
Woody
Maquis shrublands
FN
Woody
Grassland
FN
Woody
Caducifolious forest
FN
Woody
Moorland
FN
Woody
Alnus forest
FN
Woody

Figure 7