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Using easily identified macrofaunal taxa for soil zoological site assessments

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Verfasserin / Verfasser:	Christian Kantner
Studienrichtung /Studienzweig (lt. Studienblatt):	A439 Zoologie
Betreuerin / Betreuer:	Ao. Univ. Prof. Mag. Dr. Alexander Bruckner

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Lebensregion
Biosphärenpark
Wienerwald

Mit freundlicher Unterstützung des Biosphärenpark Wienerwald!

„Ein Stück des Weges liegt hinter Dir,
ein anderes Stück hast Du noch vor Dir.
Wenn Du verweilst, dann nur, um Dich zu stärken,
aber nicht um aufzugeben“

(Augustinus Aurelius, 354 – 430 n. Chr.)

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Zusammenfassung

Vor über 100 Jahren wurde im Bereich der Limnologie ein Indikatorensystem für Wasserqualität entwickelt. Dazu wird für nahezu alle aquatischen Tier – und Pflanzengruppen eines Standorts das Vorhandensein erhoben. Im Bereich der Bodenbiologie sind einzelne Taxa wie Collembolen oder Laufkäfer als Bioindikatoren etabliert, die aber nur einen Bruchteil der Artenvielfalt im Boden darstellen. Eine umfassendere Untersuchung mehrerer Großgruppen der Bodenfauna scheitert fast immer an der Fülle des dabei anfallenden Materials und an taxonomischen Problemen in vielen Bodentiergruppen.

Das Ziel dieser Diplomarbeit ist es zu untersuchen ob sich ausgesuchte Taxa als indikativ für bodenbiologische Untersuchungen erweisen, die sich aufgrund einfacher Ansprechbarkeit auch von Laien bestimmen lassen.

Die wichtigsten Vorraussetzungen für die Aufnahme eines Taxons als Indikator dieser Studie waren: (i) eindeutig bestimmbar (ii) schnell bestimmbar und (iii) ohne Verwendung von Präparationstechniken und mit der Binokularlupe bestimmbar. Um dies zu testen, wurde die Bodenmakrofauna von 20 Standorten aus 4 verschiedenen Waldtypen (Buche, saure Buche, Eiche und Kiefer) sowie 5 “Sonderstandorten” (davon 2 Wiesen und 3 Schlagflächen) untersucht. Alle Untersuchungsflächen lagen innerhalb des Biosphärenparks “Wienerwald” in Niederösterreich.

Es kamen 3 verschiedene Sammelmethoden zum Einsatz: (i) Barberfallen, (ii) manuelle Handsuche am Boden und (iii) Bodenproben. Um die Differenzierungskraft der ausgewählten Taxa hinsichtlich der Standortstypen einzuschätzen, wurden Standortsparameter erhoben wie: Kronenschlussdichte, Bestandesdichte, Totholzdichte, Ellenberg’sche Zeigerwerte.

Die Resultate zeigten, dass der Faktor “*sitetype*“ (entspricht den 5 Standortstypen) einen signifikanten Einfluss auf die Ähnlichkeit der Taxa – Gemeinschaften hatte, jedoch nur 12% der Gesamtvariabilität erklären konnte. Aufgrund dessen war es nicht möglich herauszufinden, welche der Taxa indikativ für die Standortstypen sind. Von den 3 angewandten Methoden repräsentierte die “Barberfallen” Methode am besten die Artenzusammensetzungen, und zwar unabhängig von verschiedenen Datentransformationen. Diese Diplomarbeit ist eine Vorstudie, ein Endziel könnte in Zukunft ein ähnliches Indikatorensystem sein wie in der Limnologie.

Abstract

During the last 100 years, limnologists have established an indicator system which evaluates water quality by analyzing the occurrence of aquatic organisms using representative taxa from nearly all animal and plant groups. In soil zoology, single taxa like Collembola or Carabidae are established as indicators, however, evaluations are based only on a very limited fraction of animals present in the soil (taxocoenoses). A more comprehensive use of the diversity of soil organisms is hampered by tremendous identification problems in many of the groups.

The aim of this diploma thesis was to test whether single taxa - when identified to a level which can be achieved without expertise and with reasonable time effort - are indicative for soil analyses. The most important requirement for a taxon to qualify as an indicator was that it had to be identified unambigously, rapidly, and without preparation. To test this, the macrofauna of 20 sites belonging to four forest types (beech, acidic beech, oak and pine stands) and 5 non wooded sites as a contrast to the forest types (2 grasslands and 3 forest clearing sites) were sampled, all of them situated in the biosphere reserve Wienerwald in Lower Austria. Three different sampling methods were applied: (i) pitfall traps (ii) a manual search on the ground and (iii) soil samples. Taxa sampled on the sites that did not meet the requirements above were not counted. To test the indicativeness of the selected taxa, various parameters were surveyed at the sites (density of canopy, stand density, deadwood density, Ellenberg indicator values of vegetation). The results showed that the factor *sitetype* (this factor included the 4 forest types and the non-forest type) had a significant influence on the similarity of the indicator assemblages, however, contributed little to total variability (approxiamately 12%). It was not possible to analyze the influence of individual taxa on this result, due to the low explained variability. Of the three methods, the pitfall traps best represented the site assemblages, regardless of the data transformation used. In further analyses the method could be improved by more sampling dates or a overworked taxa set.

1. Introduction

Until the end of the last millennium, the concept of biodiversity was only associated with wildlife in a touristic aspect (Decaens et al., 2006). When the meeting for the "Rio Convention of Biological Diversity" took place in Rio (Brazil) in June 1992 this changed dramatically, and the term "biodiversity" (Büchs, 2003) and "monitoring" in the context of protection and conservation became very popular worldwide. Biodiversity was finally focused on the value of living species (Hagvar, 1998).

Soils are one of the most diverse habitats (with an enormous number of species) of terrestrial ecosystems. Heywood (1995) mentioned that the biological diversity in soils was much higher than the one found above the ground. However, soils were neglected in the meaning of protection over decades, and diverse human activities led to negative effects for soil organisms (Filip, 2002). Filip (1995) argued that we all should be concerned about soil protection, because only healthy, biological active soils ensure a sufficient production of food, fodder and fiber for the human population. Protection and preservation of soils in terms of soil quality became therefore an international goal (Filip, 2002).

To investigate soils regarding quality concepts, there is a variety of methods for measuring biological parameters in addition to chemical and physical surveys (Römbke and Kalsch, 2000).

Ruf et al. (2003) mentioned that biological methods are needed to assess the condition of soils. There are several concepts of soil quality, one of them is the BBSK or "biological soil classification scheme" (Ruf et al., 2003). This concept is based on two assumptions: (i) *That the composition of the soil fauna is mainly determined by the abiotic characteristics of sites* (ii) *That it is possible to find most important site parameters with the greatest influence on soil fauna* (Ruf et al., 2003, page 264). Previously selected invertebrate taxa from the soil macrofauna and mesofauna were examined to species level (in addition to several abiotic parameters) and the results showed that there were specific soil faunas for specific sites (Ruf et al, 2003). Despite this there are several other concepts for soil quality assessments based on different assumptions (e.g. Van Straalen, 1998, Filip, 2002, Barbiroli et al., 2004, Parisi et al., 2005, Sotres et al, 2005 Velasquez et al., 2007).

Ekschmitt et al. (2003) mentioned that the assessment of the soil biodiversity has to struggle with a variety of problems (i) a good taxonomic expertise, which is not guaranteed in many groups, (ii) species identifications are time-intensive and expensive.

Those problems hamper a more comprehensive use of soil organisms for monitoring and conservation concepts.

Generally speaking, due to the complexity of ecosystems (especially in soils) and the huge diversity of animals, biologists have to develop alternative methods for monitoring and conservation concepts, otherwise these tasks would be too costly and difficult to accomplish (Meffe and Carroll, 1997, Landres et al., 1988).

One possibility to overcome that dilemma is the usage of indicator taxa, which might belong to certain species or even higher taxonomic groups (Hilty and Merenlender, 2000).

The usage of soil invertebrates as bio-indicators is quite frequent, there are several approaches which use e.g. ants, beetles, collembolans, dipterans, and earthworms (Andersen and Majer, 2004, Bohac, 1999, Cassagne et al., 2005, Frouz, 1999, Rainio and Niemelä, 2003). The problem with such bio-indicators is that they are not comparable among each other, because different methods are used to collect, identify, classify, and analyze them.

Andersen (1999) mentioned that insects and other invertebrates are very appropriate bio-indicators but in reality they are mostly ignored in monitoring studies. Since species identification is expensive, several surrogate concepts have been proposed in the last decades.

One such concept in a monitoring or conservation context is the “taxonomic minimalism“ idea (Oliver and Beattie, 1993) in which taxa higher than species-level are used to simplify the identification process, and obtain rapid biodiversity assessments. Following the taxonomic minimalism concept invertebrate taxa were identified to morphospcecies or morphotypes by means of external morphological characters used by “non–specialists“ (Beattie and Oliver, 1994, Oliver and Beattie 1993, 1996). The morphospecies concept is related to the approach of appointing non-specialists, this idea is called parataxonomy. The parataxonomic approach (see e.g. Cranston and Hillmann, 1992, Bassat et al, 2000, Abadie et al, 2008) means, that untrained persons or laymen identify animals to morphospecies using morphological characters without any help of taxonomic literature or similar tools.

There are several studies which use morphospecies as surrogates for traditional species identification (e.g. Cranston and Hillmann, 1992, Derraik et al., 2002, Barrat et al., 2003).

Another surrogate approach is the higher-taxon idea, which was used in marine studies (Warwick, 1993; Roy et al, 1996; Bertasi et al, 2009) and studies on terrestrial plants, birds and mammals (Balmford et al. 1996a, Balmford et al, 1996b), and invertebrates (Andersen, 1994; Brennan et al, 2006). The underlying idea behind this approach is that the higher taxon-richness (e.g of families) reflects the richness of species.

All these different approaches have one thing in common: they identify only few groups of animals. The multi-taxon approach (Hayes, 2009, Lovell et al., 2010) might be better than the existing surrogate approaches, but – as mentioned before – it takes much time and money, when taxa were identified to species level.

In this study, I tested a new multi taxon approach for soil zoological site assessments, using the soil macrofauna. As mentioned above, this approach tried to bypass the taxonomical problems of traditional soil zoology. The questions which arose were: (i) is it possible to discriminate sites of varying character (four forest and one non-wooded types) using soil macrofauna assemblages; (ii) which of the three methods is best suited for characterizing the assemblages; and (iii) can taxa be found that are most efficient for the discrimination? An ultimate goal of this study was to set up an indicator system for soils much alike the long existing one in limnology (Kolkwitz and Marsson, 1909).

2. Materials and Methods

2.1 Sampling area

The sampling sites are located in the biosphere reserve „Wienerwald", situated in the federal lands of Lower Austria and Vienna. In 1987, the governors of the states of Vienna, Lower Austria and Burgenland signed the *Vienna Woods Declaration* to protect nature in the region. Since 2005, the Wienerwald is acknowledged as a biosphere reserve. It is Austria's largest conservation area of this kind. The bioshpere reserve „Wienerwald" encompasses an area of about 1054 km^2 and it contains more than 20 different forest types dominated by beech (*Fagus sylvatica*) and mixed oak (*Quercus spp*.) – hornbeam (*Carpinus betulus*) stands. The high diversity of different forest types can be explaind by different climatic and geographic conditions. For example one forest type, the parce downy oak wood, which is situated in dry regions of the Wienerwald contains a high diversity species such as the Eastern Green lizard (*Lacerta viridis*) or the Scarce swallowtail (*Iphiclides podalirius*). The Austrian pine stands (*Pinus nigra* are another characteristic forest type are, which are located along the thermal line in the southern parts of the Wienerwald. Due to their climatic conditions these stands are characterised by dryness and limited water supply. Within the biosphere reserve more than 17 types of meadows and 2.000 plant species can be found. The meadows are as well as the forest important for the mosaic landscape in the Wienerwald and contribute to the species diversity, e.g. nutrient poor meadows accomodate 70 plant species and 540 animal species per ha.

The biosphere reserve is divided into 3 different divisons: core regions, buffer zones and transition zones. Each zone has its own function. Core regions are characterized by nature which can develop itself without any human activity. These core regions consists only of forest regions within the biosphere reserve "Wienerwald". They are divided into 37 sectors with an area of approximately 5.000ha. Core regions are retreat possibilities for many rare species and protected by law. The second category the buffer zones contributes to the preservation of living areas, which are cultivated by human activities, such as meadows or pastures. At least the transition areas are the economy, life and relaxation area for its inhabitants.

The goals of the biosphere reserve "Wienerwald are various, to name some: (1) the protection and promotion of diversity in nature, (2) support of monitoring and research, (3) support of diversity in nature, economy and education.

In this study three different forest (five locations of each type, named e.g. site oak1, oak2….) types were examined:

(i) Oak (*Quercus petraea*), (Fig.1): concentrated around Breitenfurt (Fig.7)

(ii) Pine (*Pinus nigra*), (Fig.2): located westwards to Baden (Fig.7)

(iii) Beech stands (*Fagus sylvatica*): Two different types of beech stands were examined. Type I (named "beech") is dominated by the grass species *Carex sylvatica*, over nutrient rich pseudogley sites (Fig.3), and type II (named "acidicbeech"), is dominated by acidophilous moss species over acidic, nutrient-poor and highly draining soils. (Fig.4). Both beech Types were concentrated around Pressbaum (Fig.7).

In addition a "non-forest type" was examined, forming an "outlyer comparison" to the forest types: (iv) Three forest clearing sites, (Fig.5) and (v) Two sites of grasslands (Fig.6)

The "non-forest-type" was located around Pressbaum (Fig.7). Each site covered a sampling area of 100m^2. Five locations of each type were investigated and named e.g. site oak1, oak2 etc. In order to minimize within-type ecologcial variation, we preselected the sites of a forest type for similar age, inclination and ground vegetation.

Fig.1. Characteristics of forest type "oak" (here: site oak2) of a study on soil macrofauna assemblages and bioindication

Fig.2. Characteristics of forest type "pine" (here: site pine4) of a study on soil macrofauna assemblages and bioindication

Fig.3. Characteristics of forest type "beech" (here: site beech2) of a study on soil macrofauna assemblages and bioindication

Fig.4. Characteristics of forest type "acidicbeech" (here: site acidicbeech2) of a study on soil macrofauna assemblages and bioindication

Fig.5. Characteristics of “non forest type (here: one out of the three determined “forest-clearing sites) of a study on soil macrofauna assemblages

Fig.6 Characteristics of “non-forest type” (here: one out of the two determined grassland sites) of a study on soil macrofauna assemblages and bioindication

2.2 Sampling Methods

At each site, three sampling methods were used: pitfall traps, soil samples and manual search. Sampling was conducted in May, 2009.

2.2.1 Pitfall Traps

At each site, five pitfall traps were exposed. As shown in Fig.8, pitfall traps have been installed in the corners and in the center of each sampling area. Cups with a volume of 250ml were used and filled with a 4% aqueous formol solution.

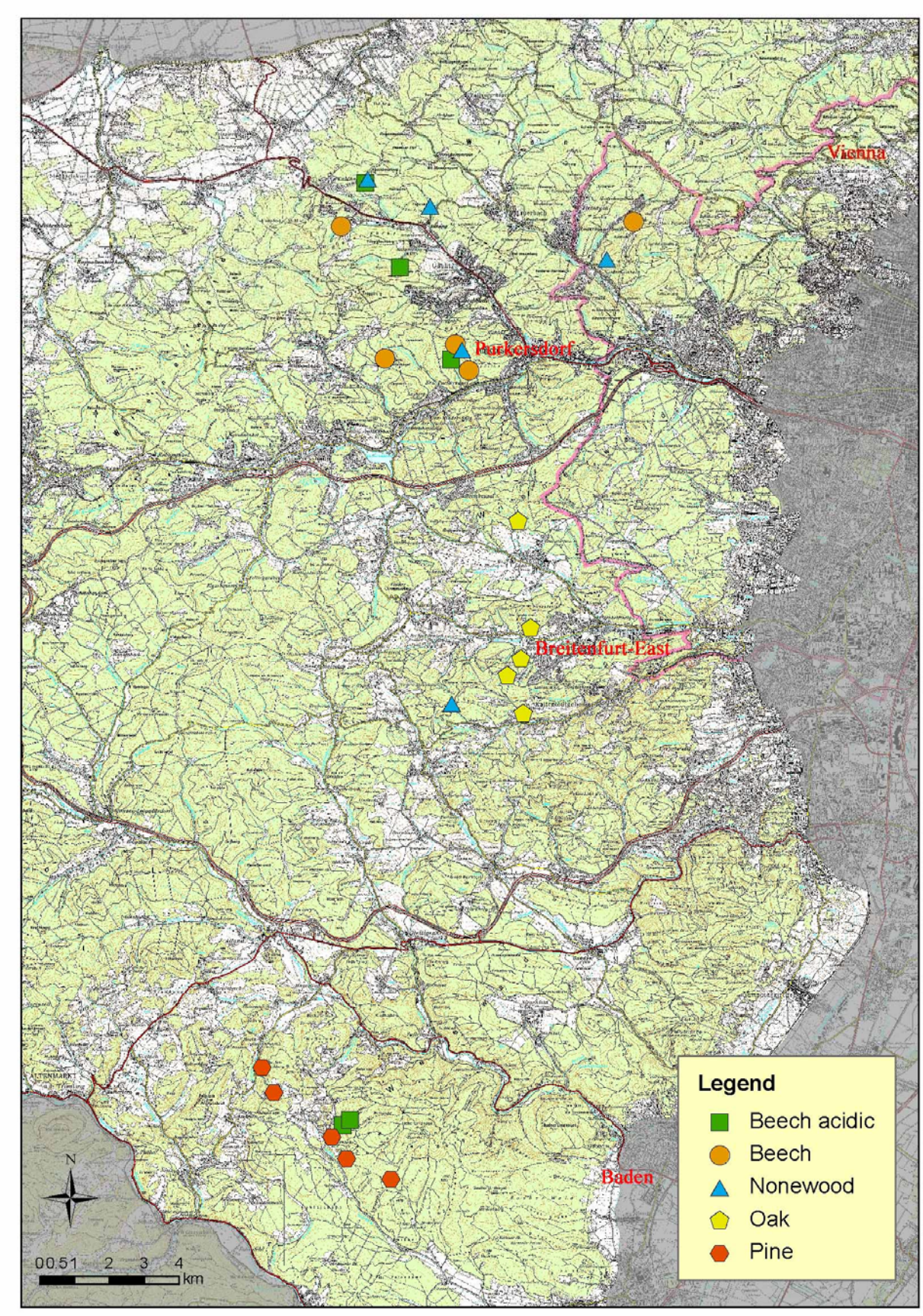


Fig.7. Overview of a map of the determined sites, green = beech acidic, orange = beech, blue nonwood, yelllow = oak, red = pine, The light yellow zone represents the area of the biosphere park Wienerwald, in which all sites were located.

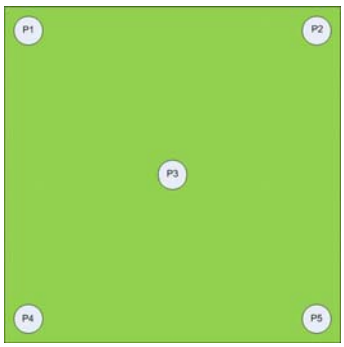


Fig.8. Scheme of the spatial arrangement of pitfall traps,
P1 – P5 represent a pitfall trap

The pitfall traps were left open for two weeks. The catch was transfered to 96% ethanol for the identification work.

2.2.2 Soil samples

At each sampling area, immediately following pitfall trap removal, we took 5 soil samples from randomized points. With a spade five 20x20cm soil monoliths were taken to a depth of 10 –15cm (including litter). The soil was passed through a Reitter sifter with a 2cm mesh (Fig9 and 10). The troughfall were put into plastic bags, transferred to the laboratory, and searched for dead or living animals for a maximum of 5 minutes per sample.

2.2.3 Manual search

After pitfall removal, we manually searched the sampling plots for a maximum of 10 minutes, especially under stones and down wood.

Fig.9. The use of a Reitter sifter for soil samples

Fig.10. Metal grid inside the Reiter Sifter

2.3 Site parameters

2.3.1 Inclination / Exposition / GPS Coordinates

Expositon, geographic coordinates as well as the height above sea level for each site was determined with a handhold GPS for each site. The inclination was measured with a goniometer.

2.3.2 Density of canopy

To assess the density of canopies, photos were taken with a fisheye lens (Nikon D200). These photos (see Fig.11) were deskewed with Photoshop CS3, converted to a black-and-white image, and the area of open sky determined to get an estimate of the light conditions at the sampling plots.

Fig.11. example of a photo with a fisheye lens from one examined beech site

2.3.3 Stand density

For measuring stand density, the Point – Centered – Quarter method (Mühlenberg, 1993) was used (Fig.12): On each site, five random points were selected. From each of these points, the distance to the nearest stem was measured in each quarter of the compass rose with a laser meter. The four measured values from each point were averaged and the stand density was calculated according to the formula:

$$D = \frac{A\,[m^2]}{\bar{d}^{\,2}_{x}\,[m^2]}$$

A = Area, $\bar{d}$ = averaged distance, D = density

For each site the 5 measured values of the stand density (according to the five points) were averaged. Additionally, the stem diameter of each measured tree was determined.

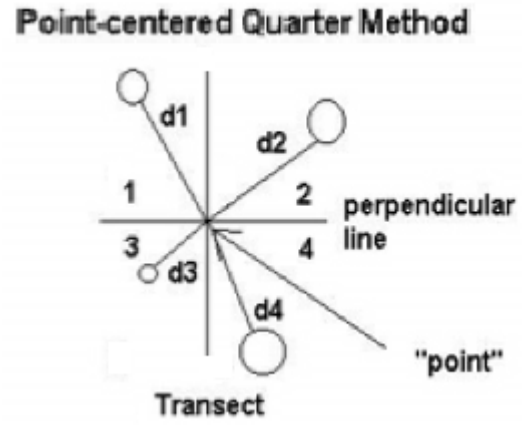


Fig.12. Scheme of the Point – Centered – Quarter method, numbers 1 - 4 represents the quarters, d1 – d4 the trees, the scheme was modified after: http://www.blackspvbiology.50megs.com/

2.3.4 Measurement of downwood

The amount of down wood was also determined with the „Point – Centered - Quarter“ method at five random points. The down wood was classified in three diameter categories: 5 – 10cm, 10 – 20cm and > 20cm.

2.3.5 Ellenberg Indicator values

On each sampling plot, the Ellenberg Indicator values were determined by a botanist according to Ellenberg, 1974. Additionally, litter depth, litter cover, vegetation cover and percentage of open ground were visually assessed.

2.4 Identification work

The identification of the collected macrofauna specimen was conducted with a binocular microscope at a magnification of 10 – 50x. The following criteria were important for the identification work:

(i) each taxon had to be exclusive on the taxa list, which means no double designations were allowed (e.g. the taxa Isopoda and Armadillidae together in the

list was not allowed, because Armadilidae is a family within the order Isopoda). This was decided on a case by case basis.

(ii) Specimens were identified according to easily and clearly recognisable characters. The characters were chosen in a way that not only trained zoologists, but also laymen should have to be able to discern these without any preparation of the specimens. As a key for identification, mainly Bährmann, 2008 was used. The identification characters according to these criteria were further used to construct an own determination key for the accuracy and practicability of this method.

(iii) The specimen were identified to that taxonomical level at which the criteria of easy and clearly recognisable characters (see point ii) were appropriate.

(iv) Taxa were not included in the taxa list, when there was potential for confusion with another similar taxa.

(v) The taxa were identified without any preparation (e.g dissection of the genitals which is often needed for species identification).

(vi) A limit of a maximum of ten minutes was set for the identification of individual specimen.

2.5 Statistical analyses

The statistical analyses were carried out with the software package Primer 6.0, including the addon package PERMANOVA.

2.5.1 Data sets and specified factors

Data sets

Two different data sets were analysed with multivariate methods: (i) the full data set included 61 taxa, wheras (ii) the reduced data set contained only 38 taxa. In the reduced data set, all taxa with <2% dominance were excluded.

Factors

Two nominal factors were specified: (i) *sitetype* and (ii) *samplemethod*. Factor *sitetype* had 5 categories, which corresponded to the forest and the non-forest site types. Both datasets were tested according to these two different factors: (i) *samplemethod,* and (iii) *sitetype*.

2.5.2 Transformations of the data

Many statistical procedures are based on the assumption of a normal distribution of the data set. In this study the data sets were not normal distributed, therefore the data were transformed. For the full data set three transformations were applied to test if different types of transformation lead to different results: (i) √√ (square root) transformation (ii) √√√√ (fourth root) transformation and (iii) presence / absence transformation. For the reduced data set only presence / absence transformation were tested.

2.5.3 Similarity measures

For the analyses of the soil macrofauna data, the Bray Curtis similarity index was used. This index is a similarity coefficient used to determine site similarities based on organism abundances. It is widely employed in multivariate analysis of assemblage data.
For the multivariate analyses of the site parameters, the Euclidian distance similarity was used. It is more appropriate for environmental parameters than for abundance data (Leyer and Wesche, 2007).

2.5.4 Multivariate analyses

PERMANOVA

The Permutational ANOVA (=PERMANOVA) analysis was performed for the underlying data sets, to find out if all groupings of the factor *sitetype* (oak, pine, beech, . .) show significant differences among each other for the given taxa samplings. The same test was used for the categories of the factor *samplemethod* (pitfall, soil sampling, and manual search).

PCA / PCO

In general, PCAs (=Principal Component Analysis) or PCOs (=Principle Coordinate Analysis) are designed to restructure, simplify and to visualize complex data sets by aggregating many variables to less meaningful Principal Components (=PC), which can be visualized in a 2-dimensional or 3-dimensional ordination plot. These Principal Components are constructed succesively in descending significance, that means the first PC accounts for the greatest amount of variation and so forth.
A PCO was used to explore the similarity among macrofaunal site assemblages, and the PCA for abiotic site parameters (e.g. litter depth). In both analyses the influences of the factors *sitetype* and *samplemethod* were tested for significance.

nMDS

Non-metric multidimensional scaling (nMDS) analyses were conducted on the soil macrofauna data of the sites. The mechanism of a nMDS analysis is that it uses the rank order of the similarity relationships between samples. The nMDS tries than to place the points in a 2-dimensional or 3-dimensional space to represent this rank order. The accuracy of an nMDS is reflected in a stress value, a formulation of the ”quality” of this representation.

CAP

A constrained ordination method, the Canonical Analysis of Principal Coordinates (=CAP) was also conducted on the data. The motivation for the CAP routine arose from the realisation that sometimes there are real differences among a priori groups in multivariate space that cannot be seen in unconstrained ordination techniques (such as PCA or nMDS), as these are designed to represent the axes of largest variability in the data set. Therefore, the purpose of CAP is to find axis through the multivariate cloud of points that are either (i) best at discriminating among a priori defined groups, or (ii) have the strongest correlation with some set of explanatory variables (Clarke and Warwick, 2001).

DISTLM / dbRDA

To find out (i) which of the site parameters significantly influenced the similarity of the soil macrofauna assemblages and (ii) how much of the total variability these parameters explain on their own, distance-based linear models (=DISTLM) were used. As modeling criterion the AICc was used, is a modification of the AIC („An Information Criterion“) that is recommended whenever the ratio N / v (N = number of samples, v = number of variables) is small (Burnham and Anderson, 2002). For visualisation of the models in an ordinaton plot, the distance-based redundancy analysis (dbRDA) was used.

3. Results – multivariate analyses

The abbrevations of the site parameters are listed in the Appendix.

3.1 Analyses of the site parameters

PERMANOVA – factor *sitetype* – site parameters

The results of PERMANOVA indicated that, the factor *sitetype* had a significant influence on the macrofaunal assemblages (Tab.1).

Tab.1. PERMANOVA results for the factor *sitetype* including information on the resemblance worksheet, the factors and the estimates of components of variation, df = degree of freedom, MS = mean squares, P (Perm) = p value, Pseudo-F = Pseudo-F ratio, SS = sum of squares, Unique perms = number of used permutations, V(Res) = residuals

Factors

Name	Type	Levels
sitetype	Fixed	5

PERMANOVA table of results

Source	df	SS	MS
sitetype	4	286.45	71.612
Res	20	217.55	10.878
Total	24	504	

Estimates of components of variation

Source	Estimate	Sq.root
S(sitetype)	12.147	3.4853
V(Res)	10.878	3.2981

PCA – factor *sitetype* – site parameters

The second analysis was a Principal Component Analysis (= PCA) which included all site parameters (abbrevations see Appendix). The results showed that: (i) the first three PC axes (PC1-PC3) explained 71.2% of the total variance. (Tab.2), and (ii) the loadings of PC4 and 5 were rather low; together they explained only 12.7% of the total variance.

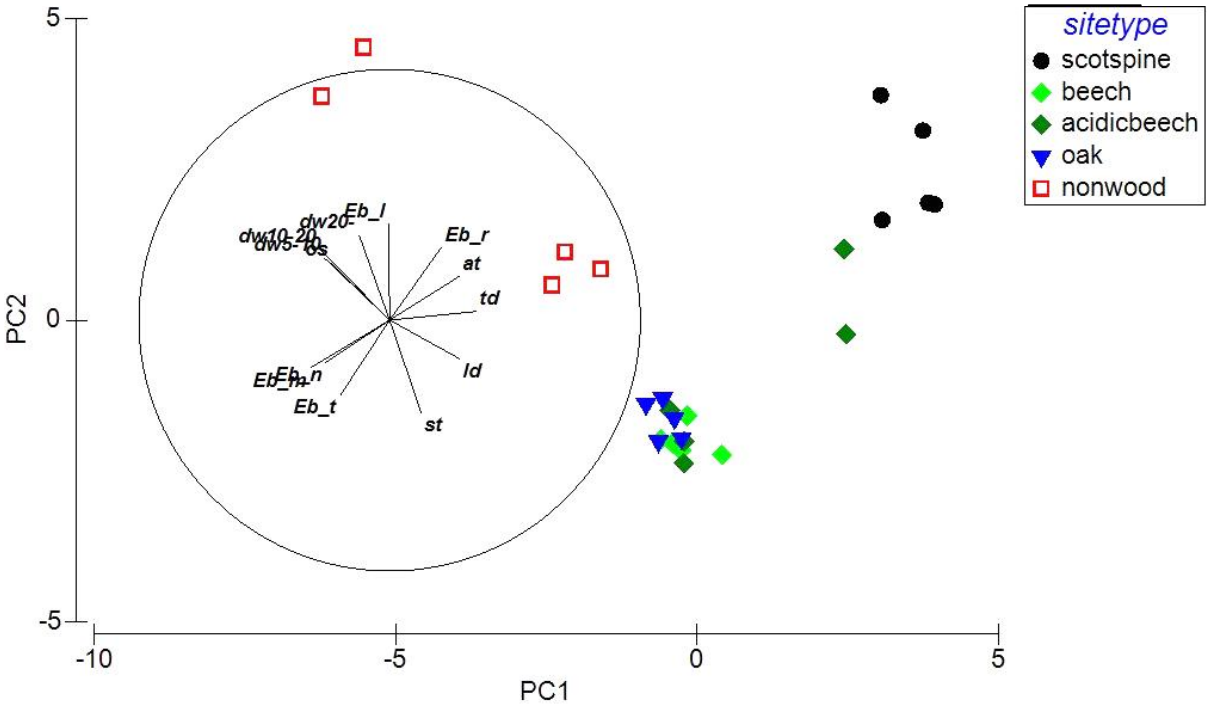


Fig.13. Two-dimensional PCA ordination plot of the site parameters from 25 soil macrofauna sampling sites. The site parameters are shown as vectors inside of the ordination plot, abbrevations see appendix.

In the 2-dimensional ordination plot, (Fig.13) two "nonwood" sites representing two grasslands clustered together in the upper left clustered together. The other three "nonwood" were "forest-clearings" and cluster a distance. All Scots pine sites tended to cluster together, whereas the acidicbeech sites showed a scattered distribution. The beech and oak sites also formed clearly definded groups, but showed some overlap however. The eigenvectors of each site parameter are listed in Tab.2.

The site parameter Ellenberg light (=Eb_l) was highly correlated with PC2, whereas the site parameter tree density (=td) with PC1 (see highlighted Eigenvectors in Tab.2)

Tab.2. Components of variability of the first five PC axis and eigenvectors of each site parameter for all PC axis, cum%Variation = cummulative % Variation, %Variation = % explained variation, referirng to Fig13

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	6.68	31.8	31.8
2	4.93	23.5	55.3
3	3.34	15.9	71.2
4	1.36	6.5	77.7
5	1.29	6.2	83.9

Eigenvectors
(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
sp_E	-0.236	-0.134	0.015	-0.363	-0.122
sp_N	-0.304	-0.183	-0.032	0.201	0.066
vc	-0.105	0.127	0.408	0.209	-0.129
og	-0.029	-0.124	-0.424	-0.033	0.194
lc	0.265	-0.077	0.330	0.074	0.155
ld	0.279	-0.154	0.184	0.079	0.276
Eb_l	-0.002	**0.383**	-0.085	-0.038	-0.391
Eb_t	-0.195	-0.297	0.218	-0.228	0.119
E_c	-0.046	0.169	0.222	-0.557	0.327
E_m	-0.316	-0.191	0.024	0.082	0.065
Eb_r	0.204	0.288	0.249	-0.085	-0.068
Eb_n	-0.254	-0.168	0.270	0.074	0.097
ot	0.092	-0.008	-0.341	0.246	0.377
ic	0.098	-0.014	-0.340	-0.394	-0.179
al	0.278	0.175	0.091	-0.143	0.341
td	**0.345**	0.034	-0.105	0.084	0.077
sd	0.127	-0.371	0.033	0.037	-0.204
dw5-10	-0.260	0.247	-0.103	-0.123	0.230
dw10-20	-0.278	0.282	0.018	-0.040	0.045
dw20-	-0.121	0.337	0.042	0.323	-0.065
os	-0.234	0.228	-0.078	0.152	0.370

CAP – factor *sitetype* – site parameters

In order to see how distinct the sites are with respect to the factor *sitetype*, a CAP analysis was conducted. The resulting 3-dimensional ordination plot (Fig.14) discriminated in five distinct cluster of all site types.

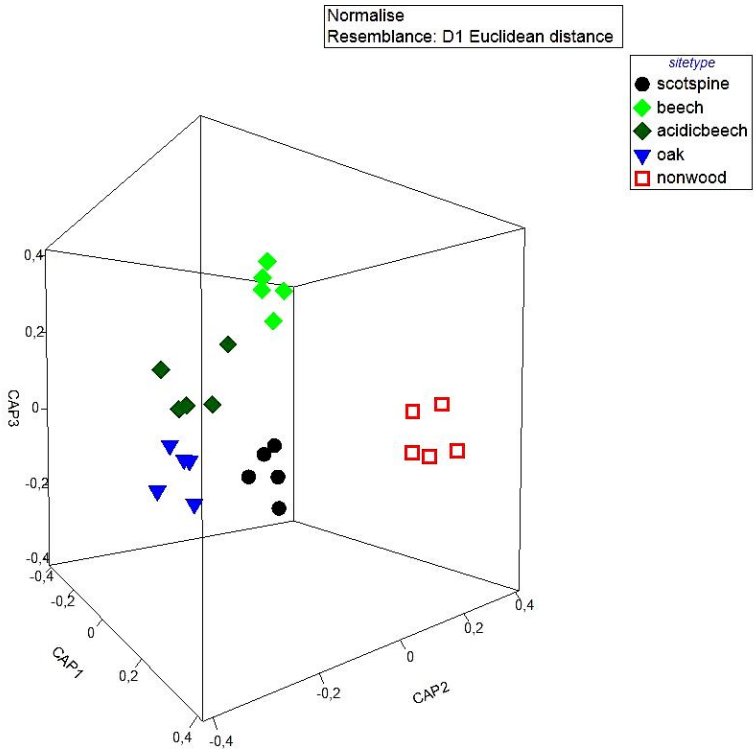


Fig.14. Three-dimensional CAP ordination plot of the site parameters from 25 soil macrofauna sampling sites; data was normalised and based on an Euclidian Distance matrix

The results showed that, all four canonical correlations were higher than 0.90 (δ_1 = 0.99, δ_2 = 0.98, δ_3 = 0.96 and δ_4 = 0.91 , Tab.3). The canonical correlations are measures for the strenght of the association between a multivariate cloud and the hypothesis of group differences (Clarke and Warwick, 2001).

The leave-one-out allocation success was quite high using this canonical model, since 22 sites out of 25 sites were correctly classified (= allocation success 88%), and only three (= 12%) were misclassified, Tab.3. The most distinct site types with 100% were the nonwood and the beech; the acidicbeech, oak and scotspine showed a success of 80%. One acidicbeech site was incorrectly classified within the oak group and vice versa. One nonwood site was mis-classified as acidicbeech (Tab.3).

Tab.3. Results of the CAP analysis concerning the site parameters from 25 soil macrofauna sampling sites, data normalised and based on Euclidian Distance similarities, shown are the Canonical Analysis and the Cross Validation; m = number of orthornormal axes used in that model

Factor for groups: sitetype

Number of samples: 25
Choice of m: 14

CANONICAL ANALYSIS

Correlations

Eigenvalue	Correlation	Corr.Sq.
1	0.9900	0.9802
2	0.9809	0.9621
3	0.9584	0.9185
4	0.9057	0.8204

Cross Validation

Leave-one-out Allocation of Observations to Groups
(for the choice of m: 14)

	Classified						
Orig. group	scotspine	beech	acidicbeech	oak	nonwood	Total	%correct
scotspine	5	0	0	0	0	5	100
beech	0	5	0	0	0	5	100
acidicbeech	0	0	4	1	0	5	80
Oak	0	0	1	4	0	5	80
nonwood	0	0	1	0	4	5	80

Total correct: 22/25 (88%)
Mis-classification error: 12%

Figure 15 showed that: (i) the two site parameters, littercover (lc) and litterdepth (ld) separated the nonwood, beech and scotspine sites from the two oak and acidicbeech sites; (ii) the nonwood sites were strongly associated with the site parameters opensky (=os), deadwood 5 – 10cm, 10 – 20cm and > 20cm; (iii): the sites oak and acidicbeech were related to the parameters stem diameter (sd) and openground (og); (iv) the parameters littercover, Ellenberg_reaction (Eb_r), and also tree density (td) were associated with the sites “beech“ and “scotspine“.

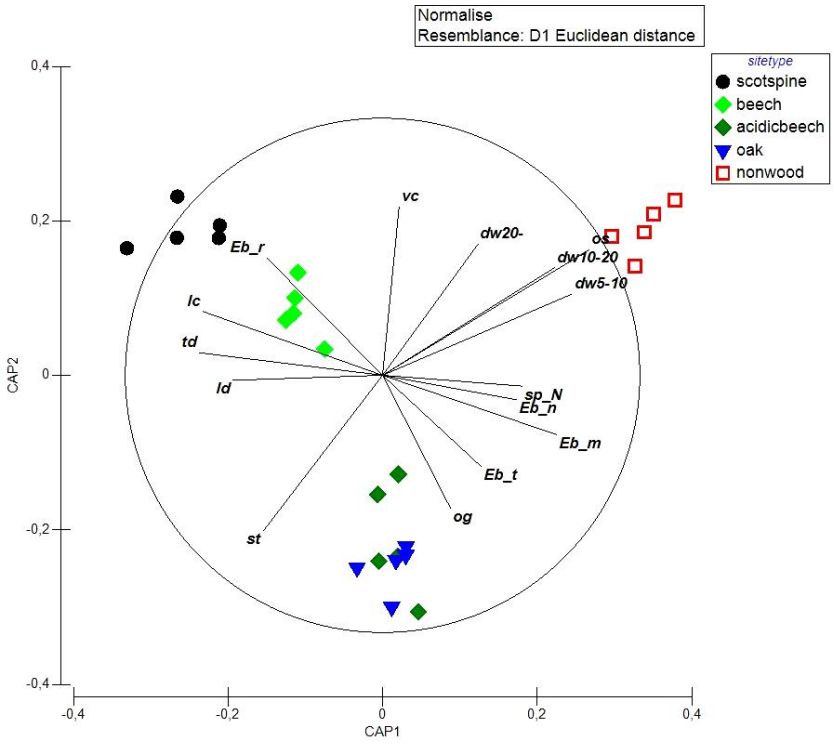


Fig.15. Two-dimensional CAP ordination plot of the site parameters of the 25 macrofauna sampling sites, site parameters are projected as a vector overlay consisting of the multiple partial correlations of the original site parameter data (normalised and transformed to Euclidian distance matrix) with the canonical axis 1 and 2

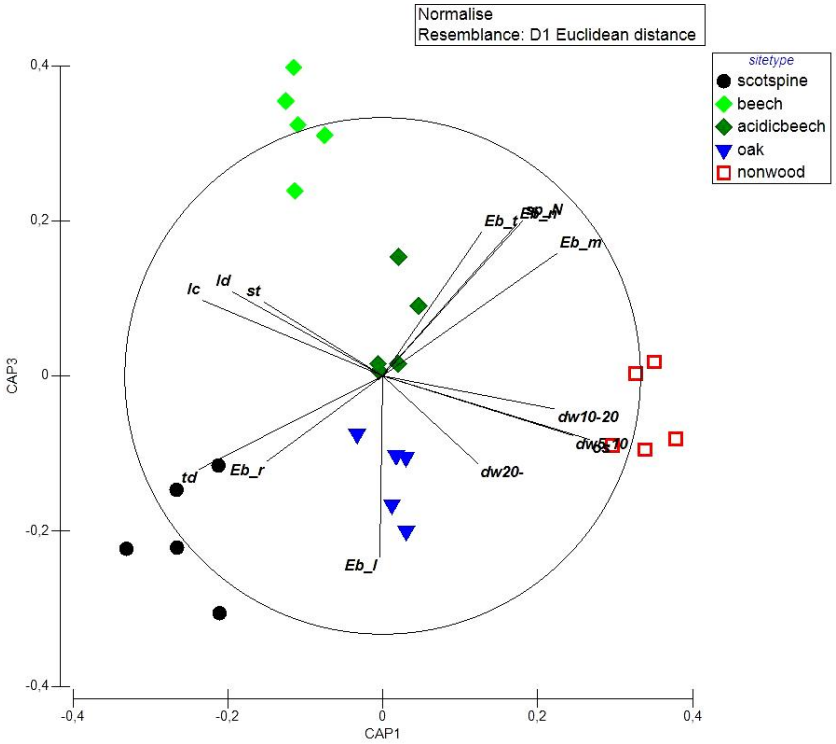


Fig.16. Two-dimensional CAP ordination plot of the site parameters of the 25 macrofauna sampling sites, several site parameters are projected as a vector overlay consisting of the multiple partial correlations of the original site parameter data (normalised and transformed to Euclidian distance matrix) with the canonical axis 1 and 3

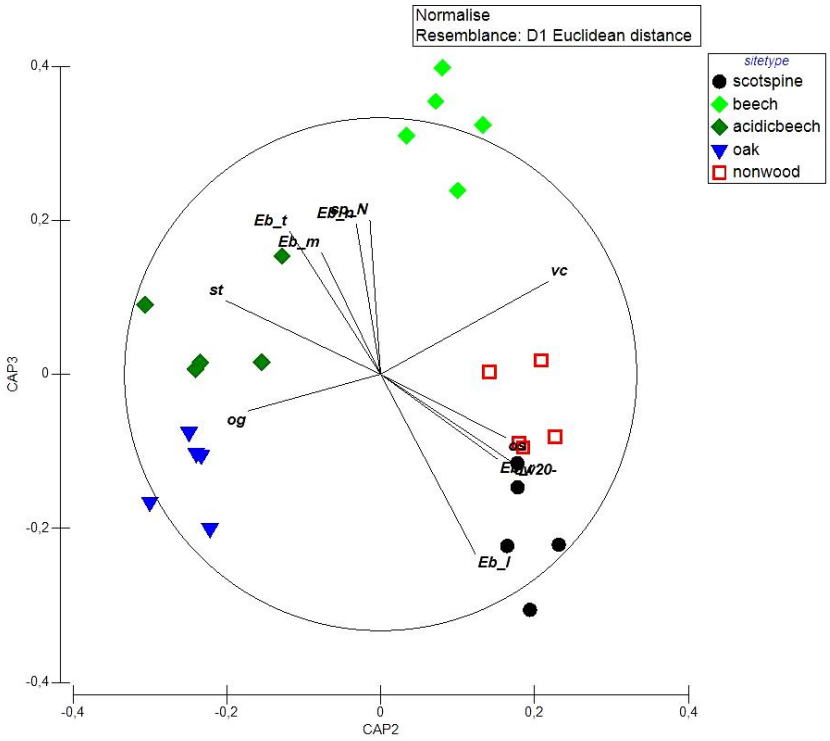


Fig.17. Two-dimensional CAP ordination plot of the site parameters of the 25 macrofauna sampling sites, several site parameters are projected as a vector overlay consisting of the multiple partial correlations of the original site parameter data (normalised and transformed to Euclidian distance matrix) with the canonical axis 2 and 3

Figure16 (CAP axis 1 and 3 shown) displayed that the nonwood sites were correlated to the parameters opensky (os), deadwood 5-10 (dw5-10), deadwood 10-20 (dw10-20) and deadwood 20- (dw20-). The site parameter Ellenberg light (Eb_l) seemed to be associated with the oak sites . This parameter was highly correlated with the CAP axis 3, and seperated the nonwood, oak and scotspine sites from the two remaining beech and acidicbeech The vectors Ellenberg light (Eb_l) and Ellenberg reaction (Eb_r) pointed in the direction of the scotspine sites.

In Fig.17 the site parameter openground (og) correlated with the oak and acidicbeech sites. Additionaly for the acidicbeech sites the parameters stem diameter (sd), Ellenberg temperature (Eb_t) and Ellenberg moisture (Eb_m) seemed to play an important role for the discrimination of these sites. As already seen in Fig15 and 16 the parameter opensky was associated with the nonwood sites. The scotspine sites were associated with the parameters Ellenberg reaction (Eb_r) and Ellenberg light (Eb_l).

Summary of the site parameter analyses

(i) The site parameters explained a high proportion of the total variance of the factor *sitetype* (more than 70% with 3 PC axes).

(ii) The CAP ordination plot revealed a clear distinction in five clusters of sites that corresponded to site types, however the results of the cross validation indicated a allocation success of only 88%.

(iii) Some site parameters seemed to have an important role in structuring the soil macrofauna assemblages.

3.2 Analyses of the full taxa set

The full taxa set contained all 61 taxa, that were chosen according to the criteria of the „Charakterkopf“ concept, as described in the Material and Methods section.

Permanova analysis – factors *samplemethod* and *sitetype*

The PERMANOVA results indicated, that:

(i) there was a statistically significant effect of the factors *samplemethod* and *sitetype* on assemblage similarity (Tab.4)

(ii) these two factors interacted with each other. Concerning at the estimates of componenents of variation (=EMS, see Tab.1), it can be noted, that 33.24% of the whole variability is represented by the factor *samplemethod* (Tab.4). Therefore, of all the factors tested, *samplemethod* had the main influence on the data variability. In comparison, *sitetype* represented only 12.90% of the total variability.

Tab.4. PERMANOVA results of 25 macrofauna sampling sites for the factor sitetype and samplemethod, including information about the analysed resemblance worksheet, the factors and the estimates of components of variation, df = degree of freedom, MS = mean of squares, P (Perm) = p value, Pseudo-F = Pseudo-F ratio, SS = sum of squares, Unique perms = number of used permutations, V(Res) = residuals

Factors

Name	Type	Levels
samplemethod	Fixed	3
sitetype	Fixed	5

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)
samplemethod	2	58461	29230	16.697	0.001
sitetype	4	16926	4231.6	24.171	0.001
samplemethodxsitetype	8	23525	2940.7	16.797	0.001
Res	60	1.0504E5	1750.7		
Total	74	2.0306E5			

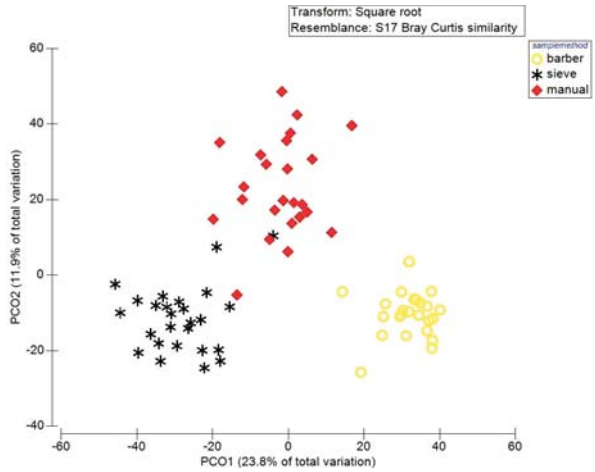
Estimates of components of variation

Source	Estimate	Sq.root
S(samplemethod)	1104.7	33.236
S(sitetype)	166.3	12.896
S(samplemethodxsitetype)	238.77	15.452
V(Res)	1750.7	41.841

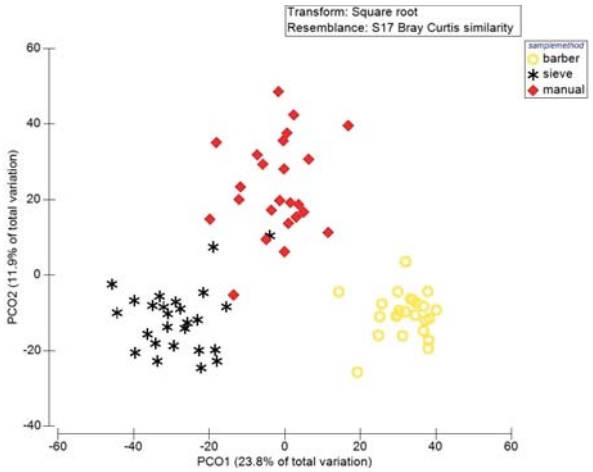
PCO analyses – factor *samplemethod*

PCO analyses revealed a separation of the data into three clusters in multivariate space, namely “manual”, “sieve” and “barber”. The cluster „barber“ tended to form a tighter group than “sieve” and “manual”, (Fig.18).

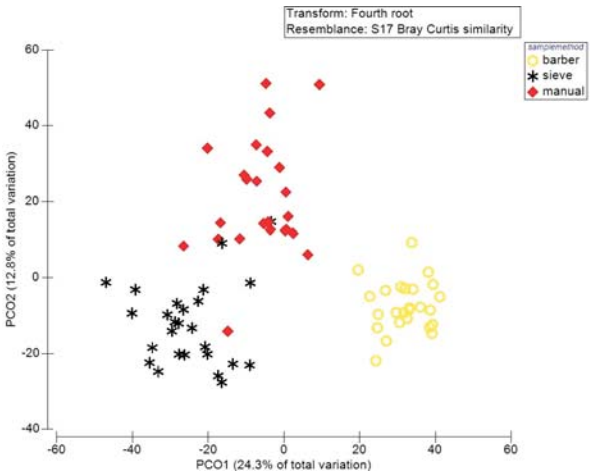
However, the variability explained by the first three PC axes of the PCO ordinations shown above (Fig18), however, revealed that, the ordination is not a good representation of assemblage similarity. PC1, PC2, and PC3=together account for only 46.41%, 44.97%, 47.57% and 51.48% respectively (see Tab.5) of the total variance for the four PCO analyses.



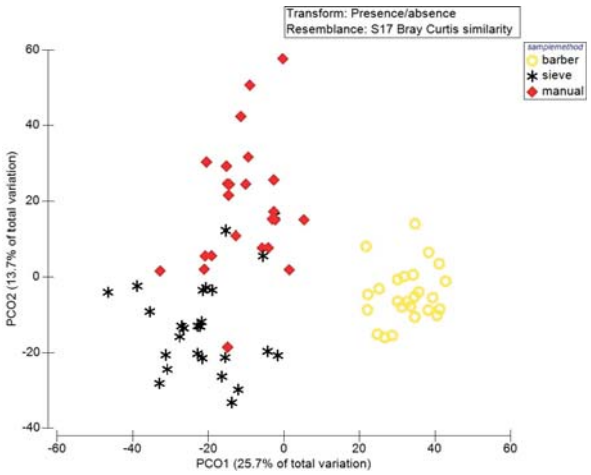
a.)



b.)



c.)



d.)

Fig.18. Two-dimensional PCO ordination plots of the soil macrofauna data, full taxa data set (a) √√- transformed and standardised, (b) √√-transformed without standardisation (c) √√√√-transformed, (d) presence / absence transformed, all PCO plots refer to the factor sitetype, PC1 (x-axis), PC2 (y-axis) and PC3 (z-axis) account for different percentage of the total variance, see Tab.5

Tab.5. Variation of explained individual PCO axes referring to Fig.18 (a) – (d)

Axis	Eigenvalue	Individual%	Cumulative%
(a)			
1	52024	25.62	25.62
2	22493	11.08	36.70
3	19726	9.71	46.41
4	17119	8.43	54.84
5	13437	6.62	61.46
6	11062	5.45	66.91
7	9904.8	4.88	71.78
(b)			
1	51068	23.76	23.76
2	25603	11.91	35.68
3	19977	9.30	44.97
4	15566	7.24	52.21
5	13123	6.11	58.32
6	10491	4.88	63.20
7	9168.3	4.27	67.47
8	9066.9	4.22	71.69
(c)			
1	47997	24.34	24.34
2	25202	12.78	37.12
3	20608	10.45	47.57
4	17067	8.65	56.22
5	13842	7.02	63.24
6	11061	5.61	68.85
7	9553.9	4.84	73.69
(d)			
1	47066	25.71	25.71
2	25100	13.71	39.42
3	22064	12.05	51.48
4	18490	10.10	61.58
5	14851	8.11	69.69

PCO analyses – factor *sitetype*

The PCO analyses described above plotted for the factor *sitetype* revealed that it was not possible to discriminate sites by this factor, when all methods (“barber”, “sieve” and, “manual”) were included in the data set (Fig.19).

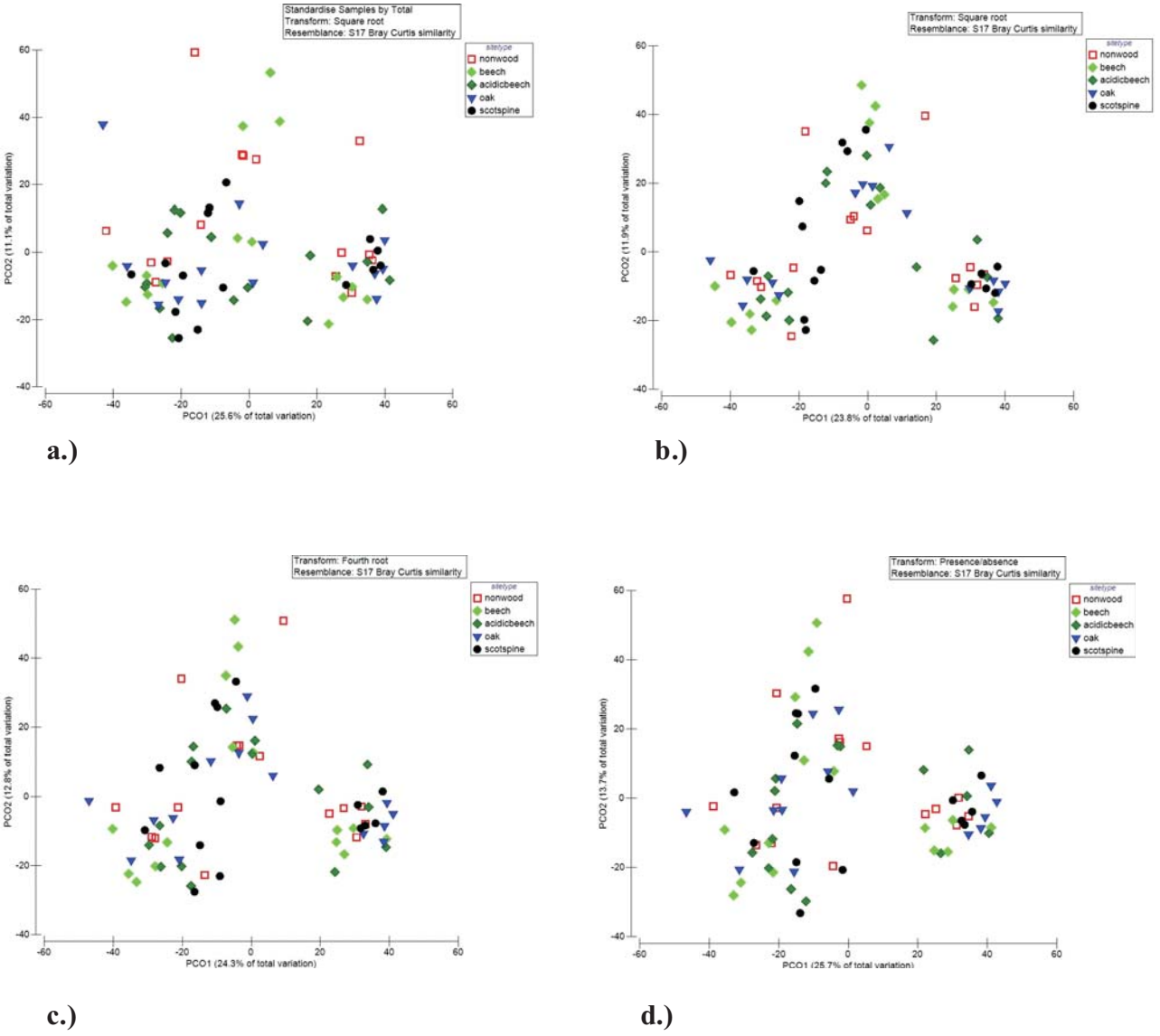


Fig.19. Two-dimensional PCO ordination plots of the soil macrofauna data, full taxa data set (a) √√- transformed and standardised, (b) √√-transformed without standardisation (c) √√√√-transformed, (d) presence / absence transformed, all PCO plots refer to the factor sitetype, PC1 (x-axis), PC2 (y-axis) and PC3 (z-axis) account for different percentage of the total variance, see Tab.5

PCO analysis – factor *sitetype* – barber data

Due to the poor PCO results for all sample methods in combination, the following analyses were confined to the barber data only.

The 2-dimensional PCO plot of the macrofauna data (Fig.20) exhibited no clear discrimination of sites due to the factor *sitetype.* The first two PC's do not explain more than 40.55 % of the total variance (Tab.6).

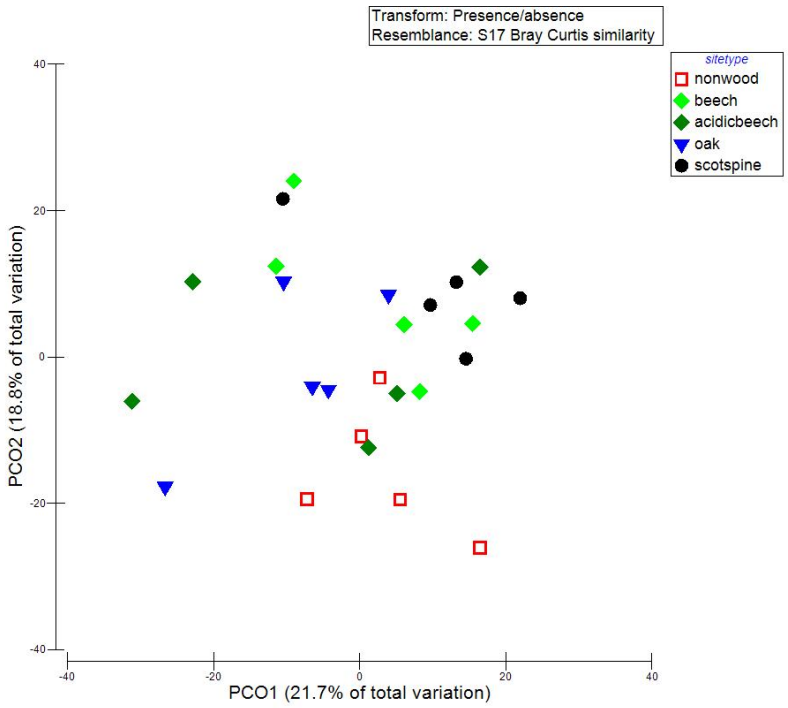


Fig.20. Two-dimensional PCO ordinations plot for the 25 sampling soil macrofauna sites for the factor *sitetype*, data based on the "barber" data set, presence / absence transformed and Bray Curtis similarities

The acidicbeech sites showed a larger scatter in the 2 – dimensional plot than the others. From the scotspine sites four out of five sites clustered closely together, while one lay off.

Tab.6. Variation of explained variability by individual PCO axis referring to Fig.20

Axis	Eigenvalue	Individual%	Cumulative%
1	4672.4	21.74	21.74
2	4042.8	18.81	40.55
3	3176.9	14.78	55.34
4	2371.4	11.03	66.37
5	2114.7	9.84	76.21

nMDS analyses – factor *sitetype* – barber data

A nMDS analysis was not successful at discriminating the different sites for the factor *sitetype*. The stress values were 0.23 for the 2-dimensional solution (Fig.21), whereas 0.15 for the 3-dimensional one (Fig.22). The acidicbeech sites showed a larger scatter, wheras four from five sites of the scotspine sites and four from five sites from the oak sites grouped closely together (Fig.21). The nonwood sites seemed to be quite well discriminated from the other sites due to the factor sitetype.

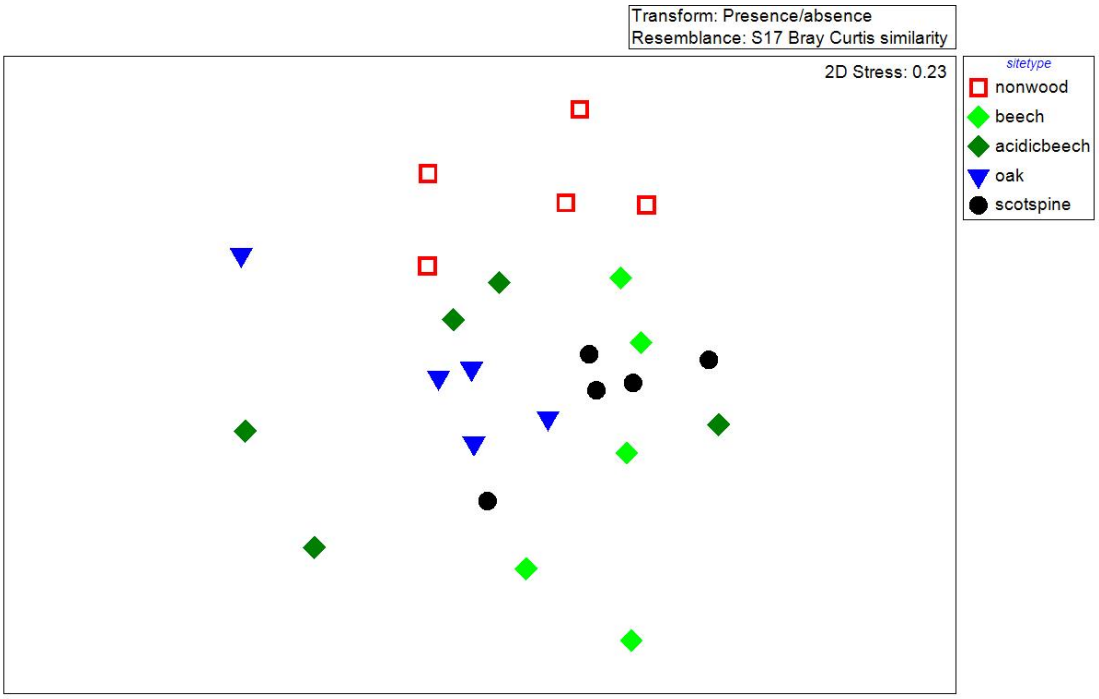


Fig.21. Two – dimensional nMDS plot of the 25 macrofauna sampling sites, based on the "barber" data set, data presence / absence transformed and Bray Curtis similarities (2d stress = 0.23)

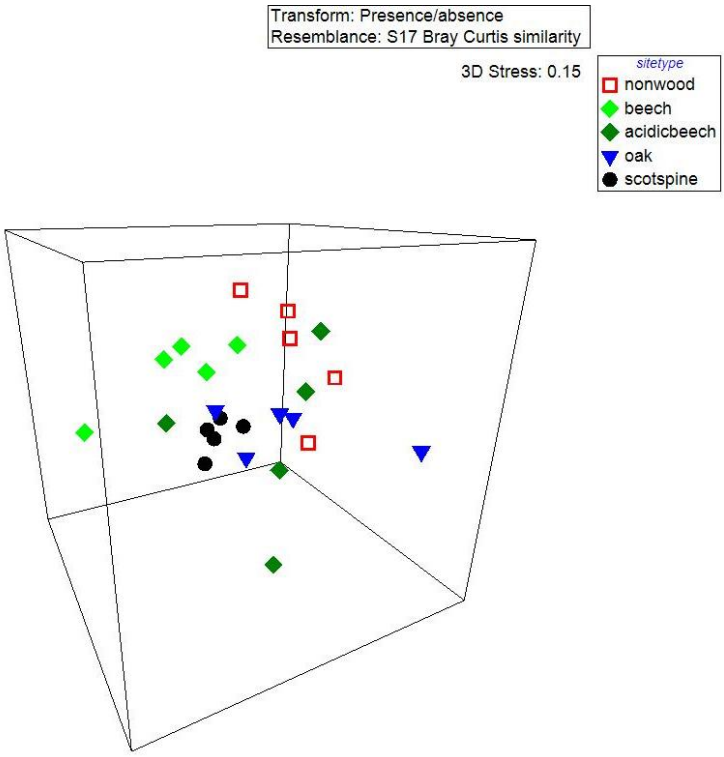


Fig.22. Three – dimensional nMDS plot from 25 macrofaun sampling sites based on the data from the "barber" method, data presence / absence transformed and Bray Curtis similarities (3d stress = 0,15)

CAP – factor *sitetype* – barber data

Based on the results of the PCO and nMDS, a CAP analyis was conducted to find out how distinct the labels concerning the factor sitetype are from each another. The constrained CAP analysis showed that most of the groups of the factor *sitetype* were indeed well distinguishable from each other. The sizes of the first two canonical correlations were high (δ_1 = 0.88, δ_1 = 0.82, see Tab.7). Number of orthonormal PCO axes were m =6.

The leave-one-out allocation success was relatively high using that canonical model: 68% of the sites were correclty classified, while 32% were misclassified. The most distinct group, which had 100% success under cross-validation, were the nonwood sites, whereas the acidicbeech sites had only 20% of success, although this group seem to be more distinct from the others in the CAP ordination plot (see Tab.7 and Fig.23).

Only one acidic beech site was correctly classified to its proper group, one was misclassified to the group nonwood, two to oak and one to the scotspine group (see Tab.7). The same applied for the group beech because this group was quite distinct from the others, but the classification success was higher (60%).

Two sites of the group beech were incorrectly classified, one as belonging to the group nonwood and another to group scotspine. The remaining ones, the groups oak and scotspine were less distinct from each other, although their classification success rates of 80% were still high. One site from the group oak was misclassified to the group beech and one site from the group scotspine was incorrectly classified to the group oak, respectively (Tab.7).

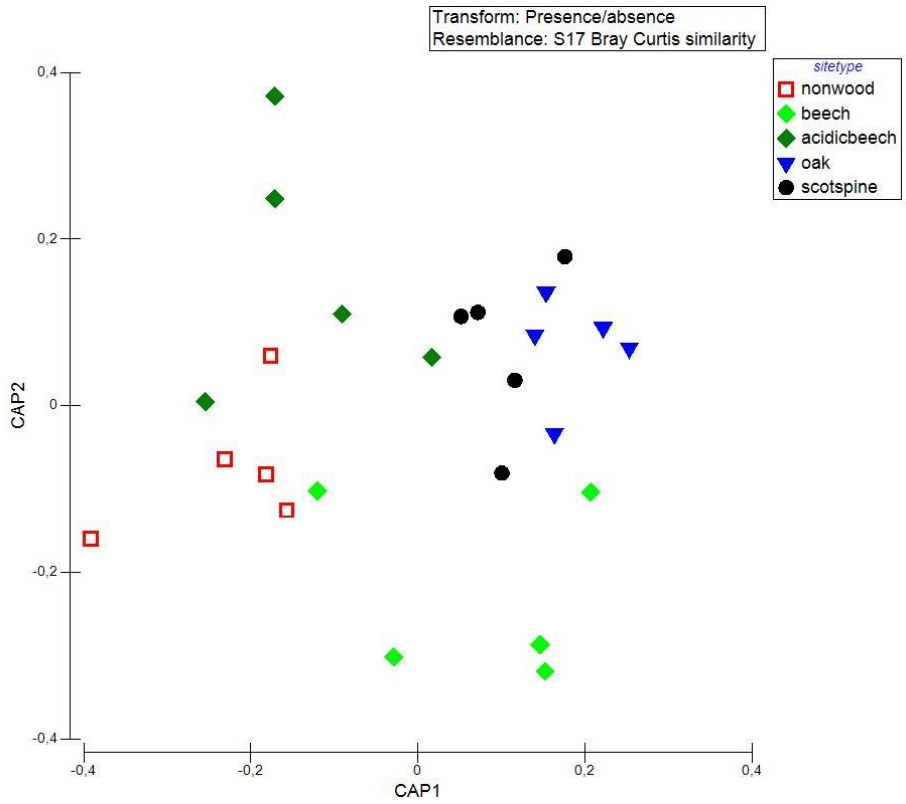


Fig.23. Two – dimensional CAP ordination plot of the 25 sites based soil macrofauna data set, only the data set from the pitfall method ("barber") was analysed, data presence / absence transformed and Bray Curtis similarities

The acidicbeech sites showed a large scatter in the 2-dimensional ordination plot. In contrast, the oak and scotspine sites tended to group closely to one another (Fig.23). The label beech sites showed a larger scatter within the 2 – dimensional solution.

Tab.7. Results of the CAP analysis of the 25 sites based soil macrofauna data, data only from the pitfall method were used in that analysis, shown are the Canonical Analysis and the Cross Validation, m = number of orthonormal axes

CANONICAL ANALYSIS

Correlations

Eigenvalue	Correlation	Corr.Sq.
1	0.8804	0.7751
2	0.8189	0.6707
3	0.7027	0.4937
4	0.6337	0.4016

Cross Validation

Leave-one-out Allocation of Observations to Groups
(for the choice of m: 6)

	Classified					
Orig. group	nonwood	beech	acidicbeech	oak	scotspine	Total
nonwood	5	0	0	0	0	5
beech	1	3	0	0	1	5
acidicbeech	1	0	1	2	1	5
oak	0	1	0	4	0	5
scotspine	0	0	0	1	4	5

Total correct: 17/25 (68%)
Mis-classification error: 32%

Summary of the analyses for the full taxa data set

(i) The PERMANOVA analysis (Tab.4) indicated a statistically significant effect on the factors *samplemethod* and *sitetype* ($P < 0.001$). *Samplemethod* explained most of the total variance (approximately 33%), while *sitetype* explained only 12%. Based on this result, I decided not to analyse which of the taxa were of significant importance as indicator for the site assemblages.

(ii) The PCO ordination plots (Fig.18) indicated that the *samplemethod* “barber“ best represented the site assemblages.

(iii) The PCO plots (Fig.19) plotted for the factor *sitetype* indicated that there is no separation among the sites for the factor *sitetype* based on the full taxa data set including all methods.

(iv) The two unconstrained analyses (PCO (Fig.20) and nMDS (Fig.21 and 22) revealed no clear separation among the labels, when the factor *sitetype* is analysed only with the data from the pitfall method.

(v) The CAP analysis (Tab.7) of the data set from the pitfall method („barber“), supplied a probably useful model (allocation-success: 68%) for discriminating the labels among the factor *sitetype*.

3.3. Analyses of the reduced data set

DISTLM – dbRDA – site parameters in combination with the soil macrofauna assemblages

The DISTLM analysis revealed that more than half of all site parameters significantly correlated to assemblage similarity (marginal tests, $p<0.05$, Tab.8). The variable littercover (=lc) explained nearly 16% of the variability in the data cloud. Other variables such as Ellenberg reaction (Eb_r), litterdepth (ld), tree density (td), and openground (og) also explained a substantial portion of the variation in the assemblage structure.

Tab.8 Results of the marginal tests from the DISTLM routine (AICc criterion) from 25 soil macrofauna sampling sites using Step-wise selection of transformed site parameters, for abbrevations of the site parameters see Appendix, P = p value, Prop = proportion of total variability, SS = sum of squares, significant parameters were highlighted ($p< 0.05$)

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P	Prop.
sp_E	1181.4	2.4309	**0.0107**	9.5589E-2
sp_N	1079.2	2.2004	**0.0251**	8.7316E-2
vc	1457	3.0738	**0.0014**	0.11789
og	1353.7	2.8291	**0.0028**	0.10953
lc	1953.5	4.3178	**0.0001**	0.15806
ld	1571.9	3.3516	**0.0006**	0.12719
Eb_l	725.91	1.4352	0.1934	5.8734E-2
Eb_t	770.05	1.5282	0.162	6.2306E-2
Eb_c	522.68	1.0156	0.4629	4.2291E-2
Eb_m	1140	2.3371	**0.016**	9.2242E-2
Eb_r	1776.6	3.8613	**0.0001**	0.14375
Eb_n	570.02	1.1121	0.379	4.6121E-2
ot	742.02	1.4691	0.1865	6.0038E-2
ic	785.76	1.5615	0.1485	6.3577E-2
at	1292.4	2.6861	**0.0048**	0.10457
td	1409.8	2.9614	**0.0014**	0.11407
sd	607.57	1.1891	0.3338	4.9159E-2
dw5-10	1115.5	2.2819	**0.0203**	9.0257E-2
dw10-20	1139.8	2.3365	**0.0162**	9.222E-2
dw20-	1102	2.2516	**0.0203**	8.9166E-2
os	1531.9	3.2541	**0.0006**	0.12395

The Step wise procedure led to a model with three site parameters (Tab.9): littercover (lc), vegetation cover (vc) and opensky (os) eplained nearly 36% of the total variance. The parameter littercover alone explained nearly 16%, followed by vegetation cover with 12%, and opensky with 8%. (Tab.9).

Tab.9. Results of the sequential tests from the DISTLM routine from the 25 macrofauna sampling sites using Step-wise selection of transformed site parameters, Cumul = Cummulative % of total variability, res.df = residuals degree of freedom, P = p value, Prop = proportion of total variability, SS = sum of squares, Variables: lc = littercover, vc = vegetation cover, os = opensky

Variable	AICc	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
lc	155.33	1953.5	4.3178	0.0001	0.15806	0.15806	23
vc	154.2	1438.4	3.529	0.0005	0.11639	0.27444	22
os	154.02	1027.4	2.7172	0.0103	8.3125E-2	0.35757	21

To visualise the resulting DISTLM model, a distance based redundancy analysis (=dbRDA) was conducted. The resulting ordination plot with an vector overlay of the three most important paramaters can be seen in Fig.23. The results revealed: (i) the first axis accounted for 44.59% of explained variability of the fitted model and 15.94% of the total variability; (ii) the second axis accounted for 34.82% of the explained variability for the fitted model and 12.45% of the total variability; and (iii) the third axis explained 20.59% of explained variability of the fitted model, and only 7.36% of the total variability (Tab.10).

A closer look on the ordination plot (Fig.23) indicated that the parameter vegetation cover (vg) was correlated with the second dbRDA axis, while littercover with the first one. Both parameters seperated the nonwood, beech and scotspine sites from the remaining ones acidicbeech and oak.

Tab.10. Percentage of % variation explained by individual dbRDA axes for the fitted model (AICc criterion) and out of total variation reffering to Fig.12

Percentage of variation explained by individual axes				
	% explained variation out of fitted model		% explained variation out of total variation	
Axis	Individual	Cumulative	Individual	Cumulative
1	44.59	44.59	15.94	15.94
2	34.82	79.41	12.45	28.39
3	20.59	100	7.36	35.76

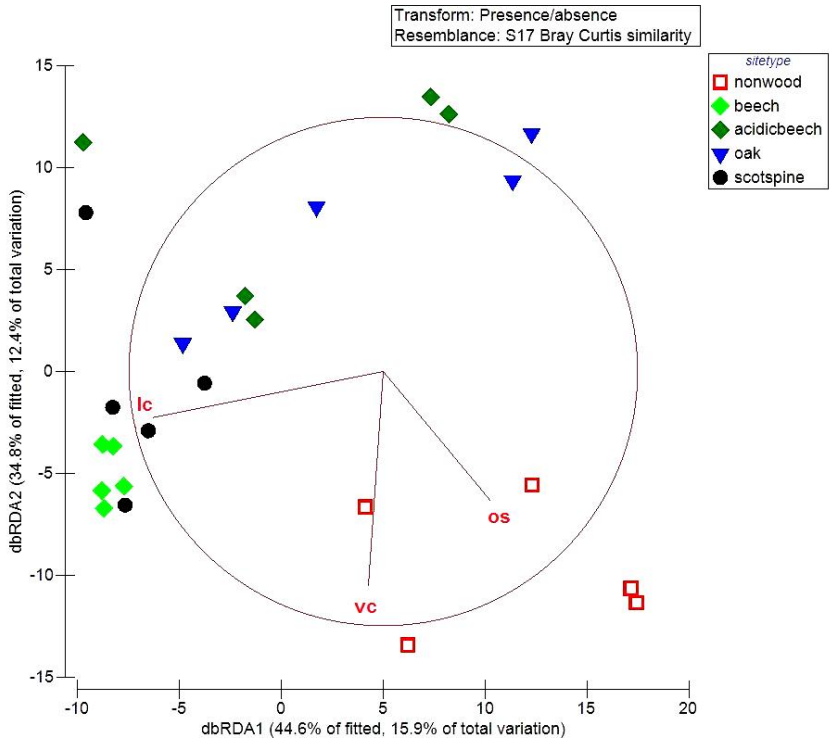


Fig.23. dbRDA ordination for the fitted model from 25 soil macrofauna sampling sites (based on Bray-Curtis after presence / absence transformation of abundances), the chosen variables for that model are projected as an vecor overlay, for abbrevations of the vector names see Appendix

PERMANOVA pair wise test – factor sitetype

To compare the groups for the factor *sitetype* among each other, a pair-wise PERMANOVA test was conducted. The results showed that all pairwise comparisons of the groups for the factor sitetype indicated a degree of significance ($p<0.05$), except the pair beech and acidicbeech (Tab.11). The group with the highest average similarity within groups belonged to scotspine (Tab.11) with 80,58%, wheras nonwood exhibited the lowest value of 69.28% .

Tab.11. Results of the PERMANOVA Pair-Wise-Tests and Average Similarity from 25 soil macrofaun sampling sites, P(perm) = p value, t = pseudo-t statistics, Unique perms = Used permutations

Factors

Name	Type	Levels
sitetype	Fixed	5

PAIR-WISE TESTS

Term 'sitetype'

Groups	t	P(perm)	Unique perms
nonwood, beech	1.568	0.0081	126
nonwood, acidicbeech	1.558	0.0172	126
nonwood, oak	16.966	0.0152	126
nonwood, scotspine	21.092	0.0072	126
beech, acidicbeech	12.841	0.1018	126
beech, oak	16.715	0.0236	126
beech, scotspine	17.502	0.0234	126
acidicbeech, oak	14.468	0.0457	126
acidicbeech, scotspine	17.859	0.0245	126
oak, scotspine	21.752	0.016	126

Average Similarity between/within groups

	nonwood	beech	acidicbeech	oak	scotspine
nonwood	69.275				
beech	66.143	70.461			
acidicbeech	65.591	68.098	69.467		
oak	66.533	67.773	68.929	73.954	
scotspine	66.634	70.603	69.758	70.377	80.575

Summary of the analyses of the reduced data set:

(i) The parameters littercover and vegetation cover pathed an important influence on the distribution of the soil macrofauna data among the factor *sitetype* (Fig.23, Tab.9)

(ii) The labels of the factor *sitetype* differ significant among each other, except the pair “beech“ and “acidicbeech“ (Tab.11).

4. Discussion

4.1 Statistical evaluation

4.1.1 Analysis of the full taxa data set

The results of the PERMANOVA test pointed out, that there are significant differences due to the soil macrofauna assemblages concerning the factors *sitetype* and *samplemethod*. Since *sitetype* explained only 13% of the total variance in the data set, we refrained from analyzing which of the tested soil macrofauna taxa are appropriate indicators for the site assemblages. The PCO analyses for the factor *samplemethod* revealed that the “barber“ samples grouped more tightly than the others. The other methods do not differentiate site assemblages so clearly. The clear distinct cluster “barber“ can be explained by the fact, that this method is not so variable as the “sieve“ and “manual“ method. This can be also explained by the fact, that the pitfalls were left open for two weeks, whereas the “manual“ and “sieve” methods were only performed once and in short time per site. Furthermore the clusters of the “manual“ and “sieve” method were not as well defined as the one from the barber method by single catches.
A closer look at the PCO ordinations however, revealed that in comparison between the different transformations the values of explained variability were quite similar. This indicated that the highly unexplained variability in the data set does not result from inadequate transformations. On that matter the study of Clarke and Warwick, 2001 demonstrated that an ordination which accounts for 70 – 75% of the original variation is considered to be a good representation of the overall structure. This value could not even be accomplished in these PCO analyses with three axes. Our analysis clearly showed that under our conditions a level of explained variability of 70% could not be accomplished with a simple 2 dimensional visualisation. The data revealed that such a value can only be obtained with six PCs. The usage of six PC’s however is not a practicable, since it can not be visualised in an ordination plot. The PCO analysis which was based only on the barber data (plotted for the factor *sitetype*) pointed out that the ordination is only a moderate representation of the underlying similarity matrix. Here, the first two PC’s did not explain more than 40.55 % of the total variance. The value of explained variability of 70-75%, which Clarke and Warwick, 2001 mentioned is considered to be a good representation, could not be accomplished in this PCO analysis.

The nMDS analyses of the factor *sitetype* also showed, that the ordinations were only a moderate representation of the underlying similarity matrix. The scatter plots, two- and three-dimensional solution, exhibited a large overlap of the site type clusters. The stress value (values < 0.1 are "good", < 0.2 "potentially useful", according to Clarke and Warwick, 2001) are 0.23 of the two-dimensional ordination and 0.15 of the three-dimensional ordination respectively.

4.1.2 Analysis of the reduced data set

The analyses of the reduced data set revealed that the site parameters littercover and vegetation cover seem to play an important role for the “Charakterkopf“ assemblages.
In general litter is very important for soil animals because (i) it represents a suitable habitat for larvaes and adults, (ii) it protects them of dehydration, (iii) it offers shelter from predators (iii), and (iv) dead plant matter is a main source of food for many soil animals.
Similar conclusions can be drawn for the parameter vegetation cover. On the basis of the low explained variability of the soil macrofauna for the factor *sitetype* it was difficult to make assumptions about the relative importance of the considered parameters. Since the parameters were only measured once at each site it is difficult to make some general statements in terms of the relative importance of these two parameters for the surveyed “Charakterkopf“ assemblages.

4.2 Possible advantages of the “Charakterkopf“ method

Gardi et al. (2009) mentioned that soil represents one of the most important reservoirs of biodiversity. For example, soils in tropical forests show a great diversity with over 2,000 soil invertebate species (Ananthakrishnan, 1996). The diversity of soil invertebrates in temperate regions is also rather high. A study in a German forest yielded 1500-1800 invertrebrate species (Weidemann, 1986).
Due to this high diversity several bioindication methods for soils were proposed, because it is not possible to identify the whole soil community, concerning costs, time and practicability. A full range of indicators were proposed, comprising either single taxa or taxocoenoses (Gardi et al, 2009). One aspect behind such indicators is that a taxon or several stands as *pars pro toto* of the whole community of a habitat. The problems with indicators in general are various due to their usage in a variety of contexts ranging from e.g identification of patterns of species

richness to the detection of ecoloical change associated with human-land use. In terms of species richness. the study of Kotze and Samway (1999) found a positive correlation between the species richness of Carabidae and Staphylinidae, but a negative between Formicidae and Araneae, Carabidae and Staphylinidae. This indicates that patterns found in one taxon may not indicate patterns in others.

Their are various problems of soil animal monitoring and indicator concepts: (1) enormous effort of processing the catch, (2) lack of qualified taxonomists, (3) inadequate knowledge of the biology and ecology of many soil animals (Cassagne et al, 2006), (4) problems with the evaluation of a useful indicator, (5) the insufficient efficiency of sampling methods and, finally, (6) the spatial and temporal variability of soil animals (Gardi et al, 2009).

The “Charakterkopf“ concept may be used to solve some of these problems. First of all the concept is extended to a multi taxa or “basket“ approach (Lovell et al, 2010, Kotze and Samways, 1999), instead to a few or one taxon. Duelli and Obrist (2003) mentioned: *the broader the taxonomic spectrum of the samples, the higher the chance of obtaining a good correlation with the entity to be assessed*.

A further advantage, and in my opinion the most important one, is the reduction of costs. Large faunal surveys and monitorings with a huge number of samples are limited by the enormous costs for the identification work. Doran and Zeiss (2000) argued that indicators of soil quality should be considered due to economic terms, both in costs and time. This is an argument against species richness as an indicator, because quantifying species richness, especially of nearly all soil taxa, means a big effort and needs qualified taxonomists which can be costly (Ekschmitt et al, 2003). Studies which focused on the costs of taxonomic work are rare, but see Vincent (1994) who surveyed the taxonomical costs in benthic macroinvertebrate monitorings or Frazzee et al. (2003) in which the costs of a floristic study of a biodiversity hotspot in South Africa were determined. In our concept, the costs were much lower than they would have been with all specimens identified to species level.

Another point is the lack of qualified taxonomists, especially for some soil zoological organism groups, which hampers the usage of many taxa for assessments. Worldwide the number of taxonomists is decreasing and the capacity is not the same in all regions. Especially in soil zoology taxonomists are rare. Our concept bypasses the taxonomical problems and lack of taxonomists by working with identification keys that enable laymen and parataxonomists to easily and rapidly process even large samples.

In comparison to other surrogate concepts, such as the higher taxon (Warwick, 1993; Roy et al, 1996; Bertasi et al, 2009) and morphospecies method (Beattie and Oliver, 1993, 1994, 1996; Derraik et al, 2002) our concept has the advantage of using more taxa than only a few which are already accepted as bioindicators, are widespread and have high abundances.

The morphospecies concept has several disadvantages compared to our concept: One is the potential overestimation and underestimation of the number of species by splitting or dumping due to much intraspecific variation, such as sexual dimorphism or large morphological differences between adult and juvenile instars (Derraik et al, 2002). Our concept has the advantage, that a taxon (as well on the species level) only gets identified when it cannot be confused with a similar taxon. This methodological characteristic prevents problems due to inter- and intraspecific variation. Morphospecies seperation is not accurate enough taxa, where even specialists have problems with species identification. This can result in a difference between morphospecies and species richness, which may be specific for an animal group.

The potential advantages of our concept are: (i) the key wich helps to easily and rapidly identify the target taxa; (ii) the high potential for saving time and cost (iii) that a broad range of taxa is used with a variety of biological and ecological features; (iv) due to the easy and manageable methods the concept could be tested in a high number of sites to compare them.

4.3 Future perspectives

Our results clearly pointed out that the sampling frequency seasonality of sampling played a critical role in such kind of studies. Concerning this, a thorough knowledge of the life cycles of the choosen taxa may be helpful to select a better period during the year to collect them. A recent study by Gardi (2009) mentioned that both spatial and temporal variability (e.g. myriapods breed several times a year) should be accounted for in monitoring concepts.

Another important point that needs to be considered regards the efficiency of the sampling techniques. Our results indicated, that the site assemblages were represented best by the “barber“ method. Pitfalls are one of the most frequently used methods in field ecology. They are often used for sampling surface-active insects (Voolma and Sibul, 2006). The advantages of using pitfalls are manifold, to mention a few: (i) simple and rapidly to install (Raworth and Choi, 2001); (ii) inexpensive; (iii) operate around the clock; and (iv) in comparison to other methods pitfalls yield large samples (Clark & Blom 1992). On the other hand there are also

some disadvantages using pitfall traps, e.g.: (i) trapping efficiency varies among several arthropod groups (Obrist and Duelli, 1996); or (ii) influence of the depth of pitfall traps (Pendola and New, 2006). Another problematic issue is the fact, that pitfall catches are biased towards mobile specimen. Concerning this method it may also be necessary to consider expanding the sampling area at the sites above $100m^2$; and also the inter-distance of the traps may be modified (see Ward et al, 2006). They were able to demonstrate that there is a significant difference between the efficiency of a 1m versus 5m and 10m distance among pitfalls. They found no differences between the 5m and 10m spacing.

On the other hand it is important to reconsider the "manual" and "soil sample" methods, although our results indicated that these were not suitable to represent the macrofaunal assemblages. Especially the "manual" method is focused on catching stages of low mobility such as larvae. Maybe these taxa of life forms are restricted to specific microhabitats and would not be detected by only using pitfall traps.

Furthermore it is necessary to proof the practicability and accuracy of this method by testing the identification key, because an important goal of this study is that non-specialists or "parataxonomists" may use it for their work. So, the key will be tested with students in the near future. Therefore it is neceassary to make sure that all students have the same basic training (Barrat et al, 2003). This will be ensured by testing biology students, who attended the same course for invertebrate identification. It is important to consider the likely variability among students, although they attended the same basic training (Barrat et al, 2003). Should new "Charakterkopf" taxa be included in the list according to the criteria specified above, the key needs to be revised.

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6. Appendix

Raw data of the abundances of the “Charakterkopf“taxa, the site parameters and the Ellenberg Indicator Values were saved as excel files. The following abbrevations were used for the sitetypes: BA = beech acidic, BE = beech, NW = nonwood, O = oak, P = pine.

Tab.12. abbrevations of the site parameters for the 25 sites bases soil macrofaunal assemblages

al	altidude
dw5-10	deadwood 5-10cm
dw10-20	deadwood 10-20cm
dw20	deadwood over 20cm
og	openground
Eb_c	Ellenberg indicator value continentality
Eb_l	Ellenberg indicator value light
Eb_m	Ellenberg indicator value moisture
Eb_n	Ellenberg indicator value nutrient
Eb_r	Ellenberg indicator value reaction
Eb_t	Ellenberg indicator value temperature
in	inclination
lc	littercover
ld	litterdepth
os	opensky
ot	orientation
sd	stem diameter
sp_E	spatial East component
sp_N	spatial North component
td	tree density
vc	vegetation cover

7. Curriculum vitae

Personal Data

Name:	Kantner Christian
Residence:	Arztgasse 73; 1220 Vienna
Email:	a0103422@univie.ac.at
Nationality:	Austria
Date of birth:	14.08.1983

Education

Sep 1989 – June 1993:	Elemantary School
Sep 1993 – June 1997:	Secondary School (Gymnasium)
Sep 1997 – June 2001:	Secondary School (Realgymnasium - Scientific focus on Biology and Physics and extracuricular classes), A-Level in June 2001
Oct 2002 – Feb 2004	Biology degree studies
Mar 2004 – Feb. 2010	Zoology degree studies

Scientific Work experience

June 2003 – Jan 2005:	Zoo Vienna
Job description:	Staff in the Guidance Group
Aug 2006:	Federal Research and Training Centre for Forests, Natural Hazards and Landscape at Mariabrunn (Lower Austria)
Job description:	Internetship in the scope within the project “Fir woolly aphids”
Mar 2010 – July 2010:	University of Natural Resources and Life Sciences
Job description:	Tutor at the field course for the lecture “Environmental and bioresourcement management”

May 2010 – Dec 2010:	BIO BIO project (http://www.biobio-indicator.wur.nl/UK/) at the University of Natural Resources And Applied Life Sciences (BOKU)
Job description:	Coordinator of the Austrian Spider Group (Field and Lab Work)

Personal skills:

Languages: Native languages: German
Other languages: English: reading, writing and speaking
Computer skills: MS Office, PRIMER 6.0+PERMANOVA, R
Others: Driving license, category B