CHAPTER 4.

ARE MACROINVERTEBRATE TAXA AND SOIL PROPERTIES SPATIALLY STRUCTURED IN DIFFERENT SUCCESSIONAL STAGES?

Introduction

Spatial and temporal dependence is widely considered to be a core characteristic of what we identify as ecological phenomena (Rossi *et al.*, 1992; Legendre & Legendre, 1998), yet in most ecological studies, as a result of the assumptions of traditional statistical tools, independence of observations in time and space is eagerly sought (Ettema & Wardle, 2002). In recent years, it has increasingly been recognised that spatially explicit patterns (formerly considered "spatial details") have implications for ecosystem function across a range of scales and are though to be an essential feature of the mechanisms that maintain biodiversity (Pacala & Deutschman, 1995).

In the last fifteen years statistical tools that investigate autocorrelated phenomena in space and time have been employed in ecological research in general (Carlile *et al.*, 1989; Rossi *et al.*, 1992; White *et al.*, 1996; Perry, 1998) and in soil ecology in particular (Robertson *et al.*, 1988; Jackson & Caldwell, 1993; Gross *et al.*, 1995; Pacala & Deutschman, 1995; Ehrenfeld *et al.*, 1997; Görres *et al.*, 1998; Saetre & Bååth, 2000; Ettema & Wardle, 2002; Chust *et al.*, 2003). The relatively new field of spatial soil ecology has lead to two general questions: (i)What are the scales at which different soil phenomena operate? (ii) is the extent of spatial heterogeneity important for soil biogeochemical processes and biological diversity?

Spatial ecological data have three characteristics: the *grain* which refers to the resolution of the data points (i.e. the space that each measurement represents); the *grain interval* or sampling interval (the separation between measurements), and the *extent*, which refers to the overall size of the study area. The *range* or the *scale* at which ecological phenomena can be considered to be spatially structured is circumscribed by the minimum and the maximum distance at which neighbouring measures are correlated. If increasing grain intervals are explored, it is often found that there is more than one scale at which a variable is autocorrelated. Therefore there are small spatial structures nested within other spatial structures at larger scales.

In such cases, the phenomenon is said to be spatially structured at several nested scales (Rossi *et al.*, 1992; Legendre & Legendre, 1998; Chust *et al.*, 2003).

Given the frequently nested character of autocorrelation, Kotliar & Weins (1990) proposed a hierarchical model for heterogeneity in ecological systems in which patches at higher scales are conformed by successive heterogeneous patches at lower scales. Consistently with this model Ettema & Wardle (2002) and Ehrenfeld *et al.* (1997) independently categorised the spatial structuring of soil organisms and soil nutrient environment respectively into three similar nested scales. Both papers propose that these scales in their turn follow the spatial patterns of physical and biological driving factors. At a scale of tens to hundreds of metres, the abundance of soil organisms and nutrient concentrations vary according to large-landscape gradients associated with human disturbance, topography, soil type and vegetation cover patchiness (Chust *et al.*, 2003; Crist, 1998; Fromm *et al.*, 1993; Robertson *et al.*, 1993). Nested within this large landscape scale, soil organisms and nutrients also display patchy distributions at the scale of centimetres to metres that seem to follow patterns associated with vegetation at the within plot-scale: plant cover, competition, species composition, growth forms and spacing (Robertson *et al.*, 1988; Jackson & Caldwell, 1993; Saetre & Bååth, 2000). Embedded within the centimetre to metre scale there is a microscopic heterogeneity that is often correlated with hotspots of decomposing organic matter and nutrient release, which are ruled by soil structure and porosity, and affect organisms in the microfood-web that operate at that scale (Anderson, 1978; Swift *et al.*, 1979).

At the within-plot scale (centimetres to metres), both the literature referring to the soil physicochemistry and that relating to soil-biota suggest a two-way spatial interdependence between the plant community and the below-ground bio-chemical heterogeneity. In one direction, spatial patterns in the distribution of soil biota and the factors that determine such patterns will influence spatial patterns of decomposition and nutrient supply (Ettema & Wardle, 2002). Heterogeneous nutrient supplies have important consequences for the primary productivity and competitive interactions of plants (Day *et al.*, 2003) that ultimately determine the structure of vegetation communities. In the reverse direction, differences in the spatial structure of plant productivity and litter supply are influential in the supply of nutrients (Jackson & Caldwell, 1993; Jackson & Caldwell, 1996 Ehrenfeld *et al.*, 1997), decomposition and soil biota (Saetre & Bååth, 2000). Therefore the spatial dimension is likely to be essential to understand the relationship between below- and above-ground processes.

The spatial structuring of soil properties and biota have been studied comparing forests and man-made ecosystems (Görres *et al.*, 1998; Saetre & Bååth, 2000; Ettema & Yeates, 2003), but rarely related to the process of secondary succession (Gross *et al.*, 1995). Yet, it seems reasonable to suggest that variation through succession in the diversity of plants above-ground will affect the heterogeneity of biogeochemical processes below-ground. Less diverse tree communities in early succession generally have larger monospecific areas and therefore the soil properties developed under the vegetation canopies are expected to be homogeneous or to form larger patches of influence. As the number of dominant species in the canopy increases with succession, not only should significant changes in means of soil properties follow (as discussed in Chapter 3), but also the spatial aggregation responsible for autocorrelation within variables is expected to increase. During secondary succession in Mexican TMCFs the number of tree species per hectare increases (González-Espinosa *et al.*, 1991; Blanco-Macias, 2001; Galindo-Jaimes *et al.*, 2002) as do the diversity of litter resources and soil macroinvertebrate taxa (Chapter 3). This chapter is the first of two steps in the investigation of the effect of secondary succession on the spatial structuring of the below-ground biogeochemical characteristics. It explores whether the increase in tree diversity per hectare through succession is accompained by an increment of the patchiness in the soil at the within-plot scale. The second step is described in Chapter 6 where I test experimentally for the cause-effect association between tree-species and soil properties.

Geostatistical methods were developed to study physical variables that are measured on continuous scales and display stationarity (Legendre & Legendre, 1998). However, counts of individuals belonging to an animal taxon are discrete and often comprise a majority of zero values. Furthermore, such counts are probably changing location continuously in space and time and therefore may not fulfil the stable spatial covariance structure assumed by geostatistical methods (Perry, 1998). SADIE (Spatial Analysis by Distance Indices) is a method developed in recent years (Perry *et al.*, 1996; Perry, 1998; Perry *et al.*, 1999; Perry & Dixon, 2002) that analyses spatial patterns in count data and does not assume stationarity or stability in covariance structures. SADIE measures the degree of nonrandomness in two-dimensional spatial patterns of populations. In this chapter variography is used to analyse spatial patterns in continuous variables such as nutrient concentrations, while SADIE is applied to determine patchiness in the distribution of taxa. Using the data obtained from sampling grids in the four successional stages of the Tarantulas chronosequence, a geostatistical analysis of the soil macroinvertebrate diversity, microenvironment, litter components and chemical variables was performed, along with a spatial analysis of the most abundant macroinvertebrate taxa. It was predicted that:

(1) The proportion of within-plot variance (coefficient of variation) would increase with succession for all variables.

(2) The number of variables with spatial structure at the within-plot scale would increase through succession. This hypothesises a significant autocorrelation for microenvironmental, macroinvertebrate diversity, chemical and litter variables (analysed with Geostatistics). For macroinvertebrate taxa abundances (analysed with SADIE) it implies an increasingly patchy distribution with succession.

(3) The size of patches for all variables would decrease with succession. For variables analysed with geostatistics this prediction implies also that the variograms of the autocorrelated variables will reach a sill at shorter distances between samples in late succession.

Methods

This chapter concentrates in the spatial analysis of the data obtained from the survey of the experimental grids in the Tarantulas Chronosequence. Details of the survey methodology can be found in Chapter 2.

Statistical Analysis

Spatial analysis of microenvironment, litter components and soil chemistry variables

A complete geostatistical procedure (variography followed by kriging) was used to analyse the spatial patterns in microenvironment, litter components and soil chemistry variables in each experimental grid. All variography procedures were performed in Variowin 3.5 and kriging and mapping were processed in Surfer 7.0 (Golden Software Inc., Golden, USA).

In order to select only those environmental variables that had considerable within-grid variation and might therefore account for a patchy distribution of the macroinvertebrate community, I analysed only those variables that had a within-grid coefficient of variation (CV) greater than 0.25. This criterion excluded completely from the analysis soil temperature, canopy cover and volumetric soil water content. Although this may seem arbitrary, the maximum within-grid range of values for these three variables was considered to be too small to cause significant changes in the local Macroinvertebrate community. It should be noted that all of the data presented in this chapter were collected in the middle of the rainy season when environmental conditions are least variable and most favourable for soil organisms, therefore a small variation around the mean was not likely to affect the local distribution of macroinvertebrate taxa. For example, the maximum variation of soil temperature within a grid was between 14.25°C and 14.84°C. This range of values is too narrow to have a significant impact on the macroinvertebrates. Although the range of values for soil water content was wider than for temperature (0.28 – 0.35 in the 15-year-old forest; table 4.1), the mean soil water content in the rainy season is high and a maximum CV of 0.21 is unlikely to limit the local availability of water for macroinvertebrates. The maximum CV for canopy cover was also small (0.07) and occurred in the 15-year-old forest (canopy cover between 84.95% and 97.74%; table 4.1). The canopy cover mainly influences soil organisms by regulating the solar radiation reaching the soil and therefore its temperature and soil water content.

Perhaps the most important evidence that the variation in soil microenvironmental conditions is not enough to drive the spatial aggregation of the macroinvertebrate community in Tarantulas comes from the findings in this chapter (see results section). Macroinvertebrate taxa were most often aggregated in the 100-year-old forest, a successional stage in which the microenvironment was not very variable (table 4.1). Conversely, macroinvertebrate taxa were rarely aggregated in the 15-year-old forest where the microenvironment was most variable.

The first step in the variographical procedure was to draw a variogram surface for each variable to determine the directions of maximum and minimum anisotropy. Then, experimental variograms were drawn for these two directions plus their two perpendiculars (with an angular tolerance of 20°) (Pannatier, 1996). The grid consisted of 49 sampling points, the minimum pair distance used was 5 m and the maximum *c.*25 m (roughly half of the maximum distance available from the data). For each point in the variograms, h-scatterplots were drawn to verify stationarity, and a multi-Gaussian distribution, to ensure that the data belonged to a single population and to locate influential outliers (Rossi *et al.*, 1992). Variables that failed to be multi-Gaussian were log-transformed. Those variables where the data consisted of a majority of zeros were not analysed because the distribution was not multi-Gaussian and no transformation is possible. In those cases where a unique data point created a set of influential outliers in the h-scatterplots, the data point was completely removed from the analysis of that variable.

Model variograms were fitted to the variables that had an initial autocorrelated phase at the 5–m grain interval in at least three of the directional variograms. It should be noted that a lack of autocorrelation between observations at the five metre scale, does not signify a complete lack of spatial pattern. It instead suggests that the spatial patterns of the variable in question operate at a finer scale than the one studied, and therefore the value at each intersection of the observed grid is not correlated with those of its neighbours.

Two-dimensional anisotropic nested models were constructed with the combination of three possible basic functions:

(1) Exponential ($\gamma(|h|) = c \cdot [1-e^{(-3|h|)/a}]$)
(2) Power ($\gamma(|h|) = c \cdot |h|^{a}$)
(3) Nugget ($\gamma(|h|) = c$)

The nugget effect was only included when it improved the fit of the model considerably. The exponential function was used when at least one of the four directional variograms had transitional behaviour and the power function when at least one of the variograms had an intrinsic behaviour. A combination of visual and statistical techniques were used to fit the best model (Pannatier, 1996). All models adopted had an Indicative Goodness of Fit (IGF) of at least 0.05 (Pannatier, 1996). Point Kriging (a form of weighted interpolation with weights based on the model variograms) was used to draw contour maps of the variables over the experimental grid. Original values were retained on the grid intersections. Contour maps were filled with a gradient of greys where the minimum value in the map is represented by white and the maximum by black. Note that in those maps in which the log-transformed variable has been plotted, the steps between contours result in greater changes in the original variable as the areas become darker. This has the effect that peaks in the original variable are sharper than they seem in the log-map.

Spatial analysis of Shannon's index of diversity

The spatial variation of macroinvertebrate taxa diversity (Shannon's index) across intersections of each grid were first explored by drawing bubble maps. Then the autocorrelation and spatial patterns of this index at the 5 m scale were analysed with a complete geostatistical procedure identical to the one described above for other variables. The same scale of greys that fill the maps was used for soil and litter to facilitate comparison.

Spatial analysis of individual taxa abundance

Due to the unsuitability of variography to analyse data in the form of counts (Perry, 1998), Spatial Analysis by Distance Indices (SADIE) (Perry, 1998; Perry & Dixon, 2002) was used to analyse the spatial arrangement of the dominant macroinvertebrate taxa in each experimental grid. Those macroinvertebrate taxa that reached a mean abundance greater than 1.5 per monolith in any successional stage (either in litter or soil) were considered as dominant. SADIE indices of Distance to Regularity (I_a) and Distance to Crowding (J_a) were calculated (using 1950 randomisations as suggested by Perry, 1998) for the dominant macroinvertebrate taxa in litter and soil for all successional stages. Tests of significance at the level of 95% confidence were performed for each index. All index calculations were performed with SADIE software created by Dr. J.N. Perry, Rothamsted Research.

For those cases where patchiness (aggregation or crowding) was significant, Clustering Indices (υi and υj) were calculated for each point in the grid, in order to determine the position of clusters and/or gaps. Kriging (assuming a linear variogram with slope 1 and anisotropy 1) was then used to draw contour maps that highlight areas with significantly higher aggregation (υi values greater than 1.5) or scarcity (υj values smaller than –1.5) than that expected at random. Mean υi and υj values were calculated for each map and corresponding randomisation tests of significance of these means performed. Because the means of υi and υj are equal to 1 and –1 when overall aggregation is similar to that expected from random, any absolute value above 1 represents the proportion of clustering or gapping in the spatial arrangement. These proportions are not equivalent to the proportion of space covered by clusters or gaps, they are a measure of the overall degree of clustering or gapping based on the frequency with which high or low abundance values neighbour each other.

Because J_a is only meaningful in cases were there is single patch of aggregation, this index was only considered when all the following conditions were met:

(1) J_a was significant

(2) I_a was not significant

(3) The υ_{ij} map showed a single patch of taxon aggregation.

Clustering (υ_{ij}) maps were only interpreted when mean υ_i and/or mean υ_j were significant or when J_a was significant (even if mean υi and/or υj were not). It is often the case that a single patch of aggregation (denoted by a significant J_a) is not enough to result in the overall pattern in the map being significantly clustered (significant mean υ_i and υ_j). To be conservative in the interpretation of patch areas, the limits of a single patch were considered to be the contours where υ_i=1.5. This area did not always coincide with the geometrical centre of the area were the abundance was higher than the grid mean (circumscribed by the contour where υ_i=0).

The formal comparison between the patterns observed in kriged maps for different variables is not a trivial matter (Perry, pers. com.; Wagner, 2003). This is probably the reason for the repeated use in the literature of visual comparisons and lack of statistical analysis to test for spatial associations between multiple kriged maps (for examples see Gonzalez & Zak, 1994; Robertson & Freckman, 1995; Görres *et al.*, 1998). A recent paper by Wagner (2003) explores the issue with new insights in multivariate spatial statistics (see the discussion section). Here the visual comparison of maps is only used as a tool for the generation of hypotheses, some of which are tested experimentally in Chapter 6.

Results

All microenvironmental variables, litter components and soil chemistry variables that had a CV greater than 0.25 are highlighted in table 4.1. Those variables that had autocorrelated phases at the 5m scale are shown in table 4.2 and the equation parameters for the fitted model variograms are presented in table 4.3. The graphed experimental and model variograms can be found in Appendix CH4 (figures CH4.1 to CH4.4)

	15-year-old			**45-year-old**			**75-year-old**			**100-year-old**		
	mean	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**
Microenvironment												
Soil temperature (°C)	14.55	0.22	0.02	13.72	0.17	0.01	13.65	0.14	0.01	13.88	0.14	0.01
Canopy cover (%)	91.35	6.36	0.07	94.41	1.53	0.02	94.87	0.94	0.01	92.67	2.64	0.03
Volumetric soil water content (cm^3/cm^3)	0.28	0.06	0.21	0.27	0.04	0.14	0.30	0.04	0.12	0.35	0.05	0.14
Litter components												
Total litter mass (g)	78.86	39.47	**0.50**	99.88	38.63	**0.39**	102.93	40.29	**0.39**	75.39	36.77	**0.49**
Unidentified (g)	23.54	16.86	**0.72**	36.16	27.54	**0.76**	47.24	25.22	**0.53**	23.10	14.55	**0.63**
Quercus (g)	0.28	0.74	**2.62**	18.25	9.81	**0.54**	21.80	11.88	**0.54**	12.70	10.08	**0.79**
Lauraceae (g)	0.02	0.14	**6.79**	0.06	0.13	**2.35**	0.22	0.46	**2.06**	1.46	1.43	**0.98**
Pinus (g)	17.91	16.21	**0.90**	14.38	19.37	**1.35**	0.72	2.15	**2.99**	0.00	0.00	0.00
other species (g)	15.11	15.97	**1.06**	3.81	2.60	**0.68**	6.09	4.49	**0.74**	9.58	5.07	**0.53**
woody and reproductive (g)	23.78	15.93	**0.67**	31.19	22.82	**0.73**	29.47	14.80	**0.50**	30.66	21.11	**0.69**
Soil chemistry												
Total carbon (%)	38.20	12.79	**0.33**	52.49	5.08	0.10	54.95	4.11	0.07	54.44	2.76	0.05
P (cmol.kg^{-1})	0.47	0.46	**0.98**	0.52	0.43	**0.84**	0.27	0.33	**1.25**	0.42	0.58	**1.39**
Mg^{++}(cmol.kg^{-1})	1.49	0.54	**0.36**	1.03	0.42	**0.41**	0.70	0.25	**0.36**	0.61	0.31	**0.52**
Na^{+}(cmol.kg^{-1})	1.78	0.83	**0.47**	1.32	0.39	**0.29**	1.19	0.41	**0.34**	0.86	0.25	**0.29**
K^{+}(cmol.kg^{-1})	8.44	5.84	**0.69**	5.98	2.13	**0.36**	2.74	1.15	**0.42**	4.00	1.27	**0.32**
Ca^{++}(cmol.kg^{-1})	3.63	2.31	**0.64**	3.87	1.61	**0.42**	1.67	0.79	**0.48**	4.26	1.83	**0.43**

Table 4.1 Mean, standard deviations (S.D) and coefficients of variation (CV) of microenvironmental variables, litter components and soil chemical composition in different successional stages. Includes measurements for all 49 grid intersections in each successional stage. Those variables that reached a variation coefficient greater than 0.25 are highlighted in bold.

Microenvironmental conditions

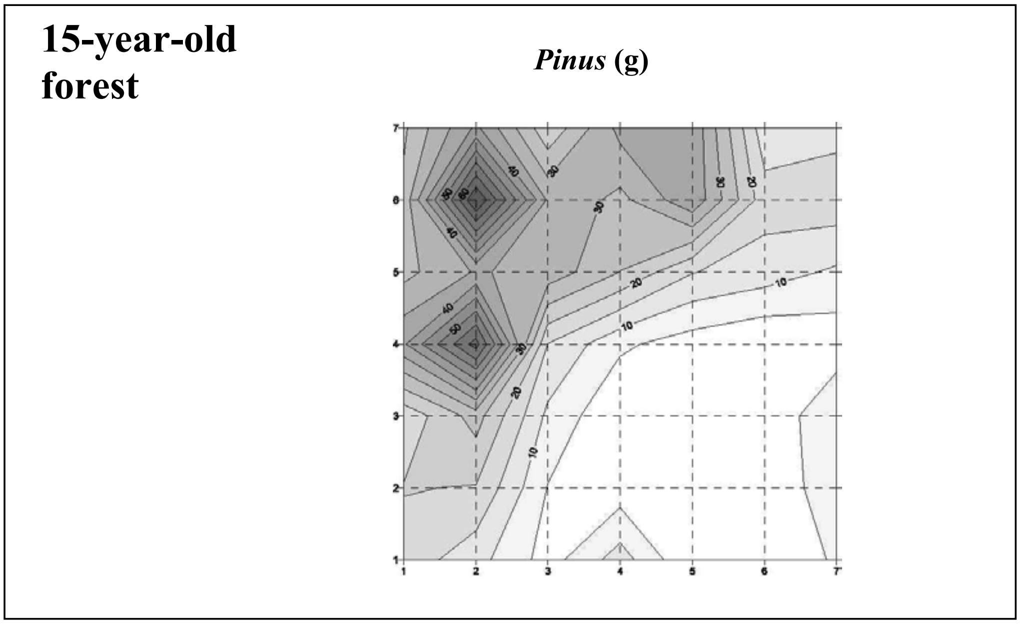
The within-grid variation of the microenvironmental variables (soil temperature, volumetric soil water content and canopy cover) was considered to be too small (CV<0.25; table 4.1) to account for major spatial patterns in the macroinvertebrate data and therefore their spatial patterns were not analysed.

	15-year-old	**45-year-old**	**75-year-old**	**100-year-old**
LITTER COMPONENTS				
Total litter mass (g)	n.a.	n.a.	n.a.	*
Unidentifiable (g)	n.a.	n.a.	*	*
Quercus (g)	---	*	*	*
Lauraceae (g)	---	---	n.a.	*
Pinus (g)	*	*	*	---
Other species (g)	n.a.	*	*	n.a.
Woody and reproductive (g)	n.a.	*	n.a.	*
SOIL CHEMISTRY				
Total carbon (%)	*	---	---	---
P (cmol.kg^{-1})	*	*	n.a.	*
Mg^{++}(cmol.kg^{-1})	*	*	n.a.	*
Na^{+}(cmol.kg^{-1})	*	*	n.a.	*
K^{+}(cmol.kg^{-1})	n.a.	n.a.	*	n.a.
Ca^{++}(cmol.kg^{-1})	*	n.a.	*	*
SHANNON'S DIVERSITY INDEX				
Litter	n.a.	n.a.	n.a.	*
Soil	n.a.	n.a.	n.a.	*

Table 4.2 Summary of the results obtained from the variography performed on the litter components, soil chemistry variables and Shannon's Index of macroinvertebrate diversity. * indicates those variables that had variograms with autocorrelated phases in at least three directions, n.a. indicates those variables that had no autocorrelated phase at the 5 m scale and --- indicates variables that had a variation coefficient smaller than 0.25 or that consisted of a majority of zeros.

Litter components

The abundance of all the litter components in all successional stages had CVs greater than 0.25 and therefore were considered for the spatial analysis (table 4.1). However, in the 15-year-old forest *Pinus* needles were the only litter component that had an autocorrelated phase at the 5 m grain interval (table 4.2). In this forest there was a gradient of increasing abundance of pine needles across the grid between the corner intersections (7,1) and (1,7) (figure 4.1). In both the 45- and 75-year-old forests, four litter components had autocorrelated phases (tables 4.3; figures 4.2 and 4.3). The spatial patterns of the litter components within the grids of these successional stages differed from one another (figure 4.2). In the 100-year-old forest, the total litter mass, unidentifiable material and woody and reproductive material were autocorrelated and had very similar spatial patterns of abundance (figure 4.4). In this same successional stage however, the abundances of *Quercus* and Lauraceae leaves were also autocorrelated but their spatial patterns did not resemble any other component (figure 4.4).



15-year-old forest

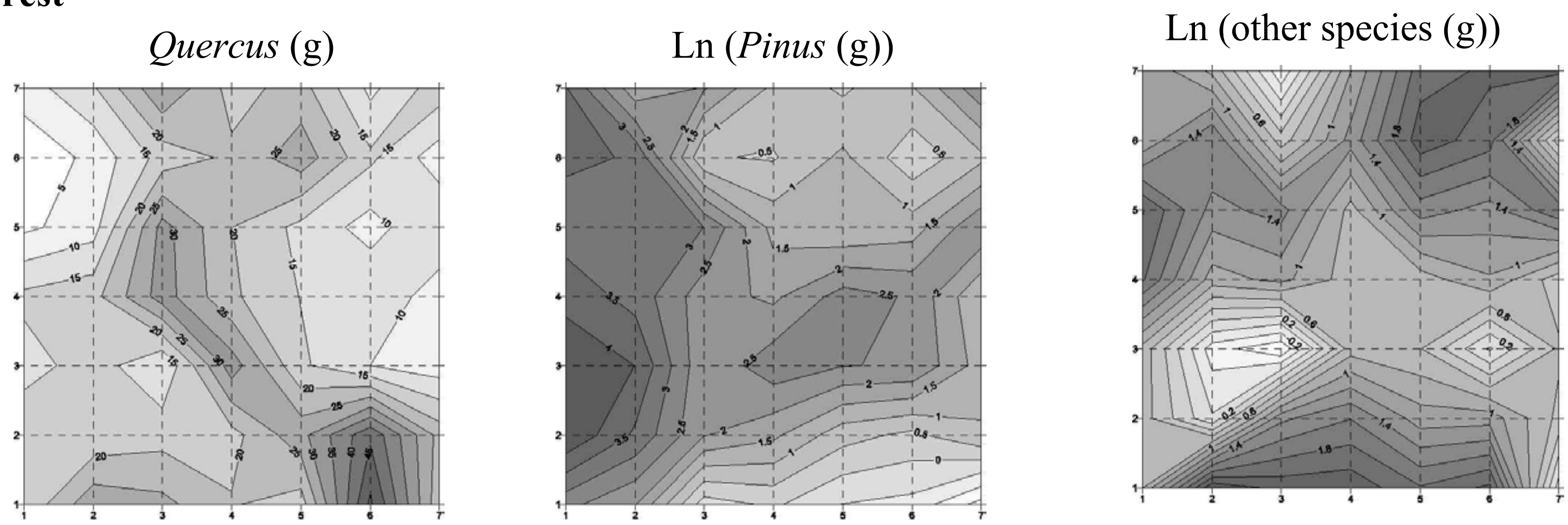
***Pinus* (g)**

Figure 4.1 Kriged contour map of pine needles mass in the litter of the 15-year-old forest. One unit on the grid is equivalent to a distance of 5 m.

Soil chemistry

All soil chemistry variables except total carbon had CVs greater than 0.25 in all successional stages (table 4.1). Total carbon had a CV greater than 0.25 and was autocorrelated (table 4.3) only in the 15-year-old forest grid. In this forest its concentration varied considerably across the grid (figure 4.5). In this same successional stage, concentration of magnesium and calcium ions had very similar spatial patterns across the grid; they were generally low in concentration, but relatively high in the half delimited by the 4 and 7 x co-ordinate lines (right half in the map in figure 4.5). Their concentration was particularly high in the area surrounding the co-ordinate (7,2). Concentration of phosphorus and sodium ions had similar patterns in that their highest concentrations were located in the half of the

45-year-old forest



Quercus (g)

Ln (*Pinus* (g))

Ln (other species (g))

Woody and reproductive material (g)

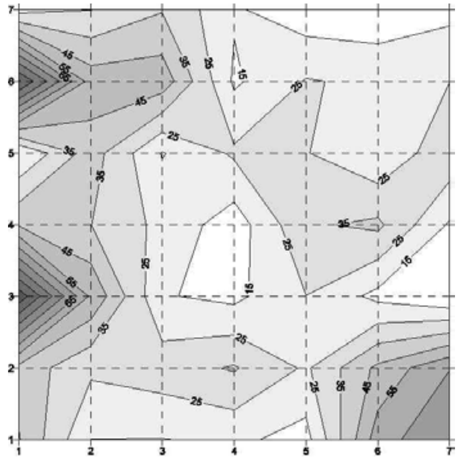
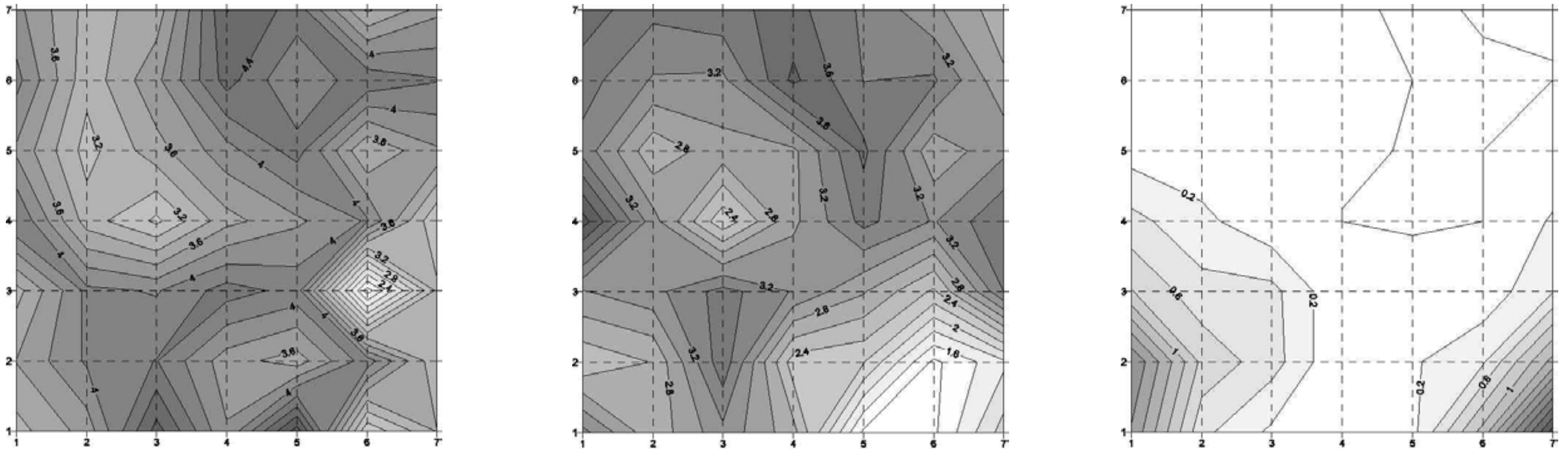


Figure 4.2. Kriged contour maps of litter components that had an autocorrelated phase in the 45-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

75-year-old forest


Ln (unidentifiable material (g)) Ln (*Quercus* (g)) Ln (*Pinus* (g))

Ln (other species (g))

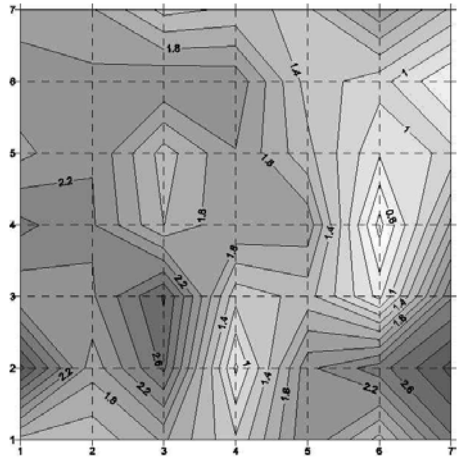
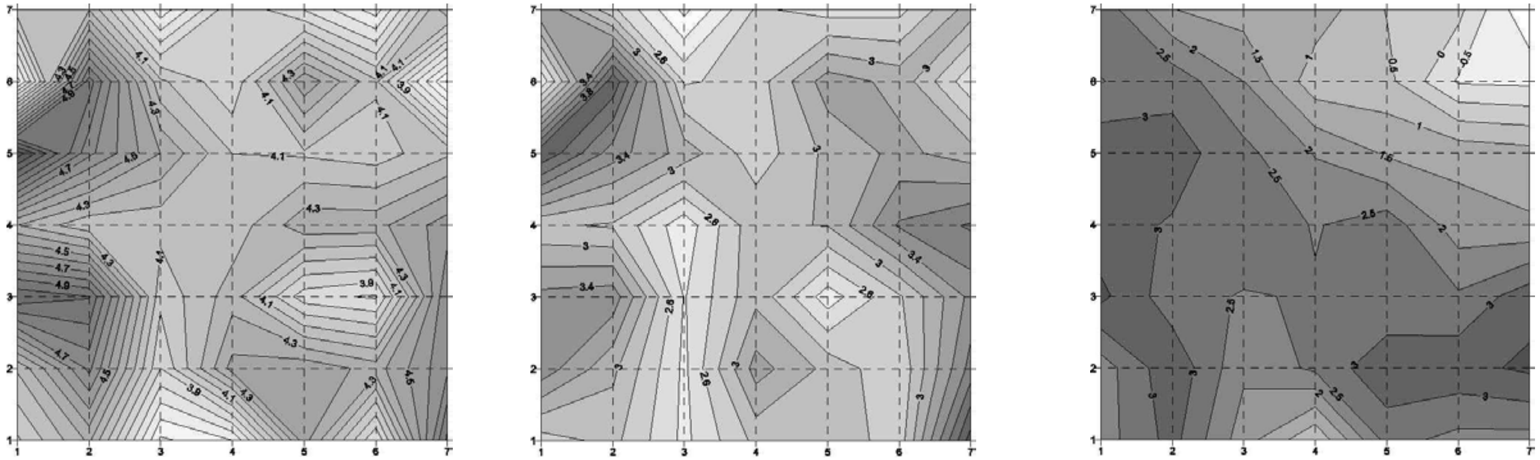


Figure 4.3 Kriged contour maps of litter components that had an autocorrelated phase in the 75-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

100-year-old forest

Ln (total litter mass (g)) Ln (unidentifiable material (g)) Ln (*Quercus* (g))



Ln (Lauraceae (g)) Ln (woody and reproductive material (g))

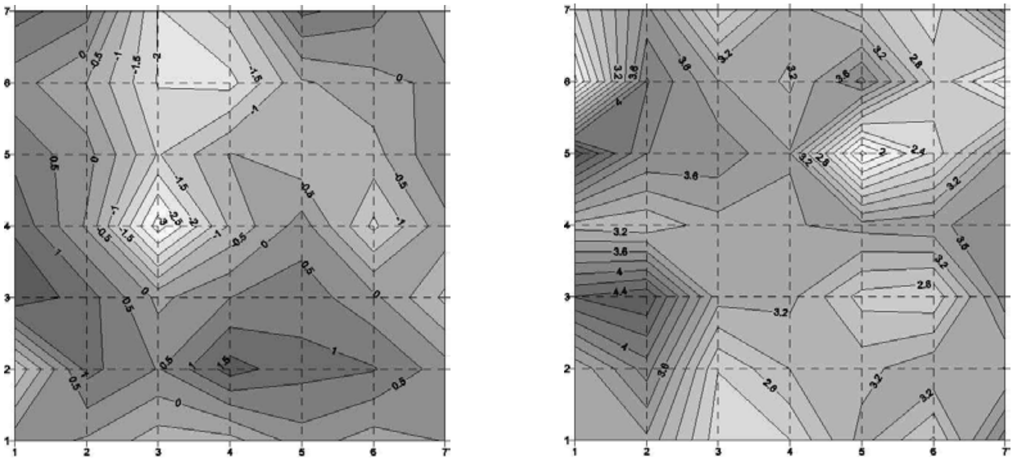
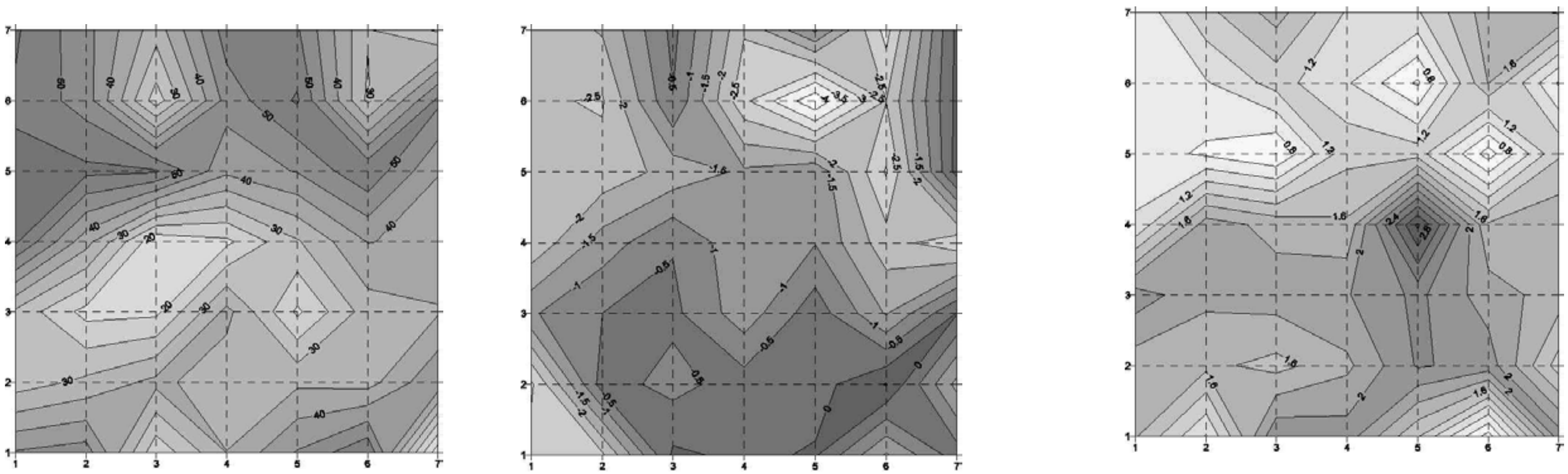


Figure 4.4. Kriged contour maps of litter components that had an autocorrelated phase in the 100-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

	First model					**Second model**					**Nugget effect**	**GOF**
	type	**C**	**a**	**angle**	**aniso-tropy**	**type**	**C**	**a**	**angle**	**aniso-tropy**		
15-year-old												
Litter components												
Pinus (g)	Power	50.61	0.63	38.2	0.55	Power	43.74	0.90	135	1000	0	0.024
Soil chemistry												
Total carbon (%)	Exp.	139.32	2.51	45.0	0.74	Exp.	44.24	4.59	135	3.7	0	0.048
Ln P (cmol.kg^{-1})	Power	0.39	0.66	53.0	2.3	Exp.	0.66	0.58	174	3.1	0.16	0.032
Mg^{++}(cmol.kg^{-1})	Power	1.10	0.49	45.0	0.67	Power	1.27	0.01	135	4.8	0.16	0.031
Na^{+}(cmol.kg^{-1})	Power	0.10	0.33	35.0	0.61	Power	0.07	0.54	91	1000	0	0.038
Ca^{++}(cmol.kg^{-1})	Power	5.77	0.93	153.0	73	Exp.	14.57	0.468	51	11	0	0.020
45-year-old												
Litter components												
Quercus (g)	Exp.	59.33	1.69	31	1.9	Power	4.06	1.9	157	82	0	0.038
Ln Pinus (g)	Exp.	0.94	4.03	90	4.7	Power	0.50	0.98	180	3.8	0	0.025
Ln other species (g)	Power	0.04	0.96	63	95	Exp.	0.51	1.56	111	4	0	0.054
Woody and reproductive (g)	Power	76.30	0.61	50	0.62	Power	68.29	0.45	140	300	0	0.012
Soil chemistry												
P (cmol.kg^{-1})	Power	0.05	0.70	23	2.70	Exp.	0.05	0.05	135	4.6	0	0.031
Ln Mg^{++}(cmol.kg^{-1})	Exp.	1.02	0.40	90	0.75	Exp.	1.51	2.96	180	4.4	0	0.024
Ln Na^{+}(cmol.kg^{-1})	Exp.	0.09	1.17	90	0.17	Power	0.03	0.54	180	1.1	0	0.040
75-year-old												
Litter components												
Ln Unidentifiable (g)	Exp.	0.12	2.54	130	0.08	Exp.	0.13	3.28	40	0.96	0	0.036
Quercus (g)	Power	16.50	1.13	90	3.5	Exp.	66.54	1.32	180	2	0	0.027
Ln Pinus (g)	Exp.	0.21	5.1	180	2.1	Power	0.01	1.74	90	2.1	0	0.038
Ln other species (g)	Exp.	0.35	1.43	147	78	Power	0.02	1.47	22	33	0	0.051
Soil chemistry												
Total carbon (%)	Exp.	4.56	0.63	135	4.9	Power	1.65	0.90	45	50	0	0.014
K^{+}(cmol.kg^{-1})	Exp.	0.08	2.20	45	0.75	Power	0.01	1.2	135	1.3	0	0.013
Ln Ca^{++}(cmol.kg^{-1})	Exp.	0.07	0.74	90	1.6	Power	0.02	1.07	180	3.9	0	0.029
100-year-old												
Litter components												
Ln Total litter mass (g)	Exp.	0.11	0.26	45	0.66	Power	0.04	0.6	135	1.3	0	0.027
Ln Unidentified (g)	Exp.	0.18	0.33	45	0.53	Power	0.06	0.8	156	29	0	0.038
Ln Quercus (g)	Exp.	0.32	3.81	135	1.8	Power	0.29	1.39	45	3.3	0	0.022
Ln Lauracea (g)	Exp.	0.44	2.98	62	1.1	Exp.	0.86	3.22	154	1.1	0	0.046
Ln woody and reproductive (g)	Exp.	0.28	0.53	90	4	Power	0.09	0.31	180	1.7	0	0.041
Soil chemistry												
Ln P (cmol.kg^{-1})	Power	0.32	0.59	45	4.6	Exp.	0.55	0.44	135	0.23	0	0.029
Ln Mg^{++}(cmol.kg^{-1})	Exp.	0.10	0.95	135	0.25	Power	0.03	0.64	45	0.71	0	0.024
Ln Na^{+}(cmol.kg^{-1})	Exp.	0.10	1.17	87	1.7	Power	0.02	1.2	30	59	0	0.047
Ln Ca^{++}(cmol.kg^{-1})	Exp.	0.05	0.89	117	0.93	Power	0.01	0.98	148	2.5	0	0.033
Shannon's diversity index												
Litter	Exp.	0.15	0.87	203	0.35	Power	0.03	0.98	71	2.5	0	0.024
Soil	Exp.	0.04	1.31	184	1.2	Power	0.01	0.96	57	4.6	0	0.025

Table 4.3 Nested models fitted to four-directional experimental variograms of litter components and soil chemistry variables in different successional stages. Only variables that had an autocorrelated phase are presented. Ln precedes the names of those variables that were log-transformed to meet distributional assumptions. Goodness of fit (GOF) is presented in the last column.

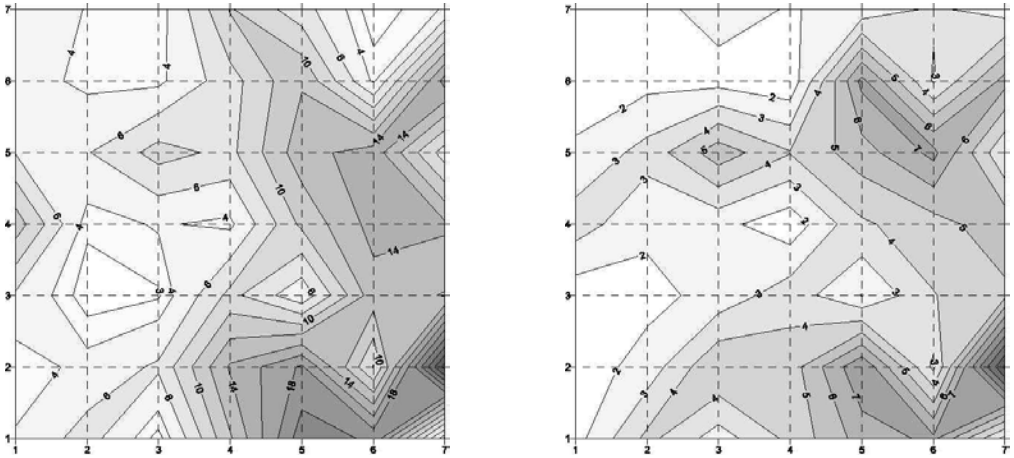
15-year-old forest



Total carbon (%)

Ln (Total phosphorus (cmol.kg^{-1}))

Na^{+} (cmol.kg^{-1})

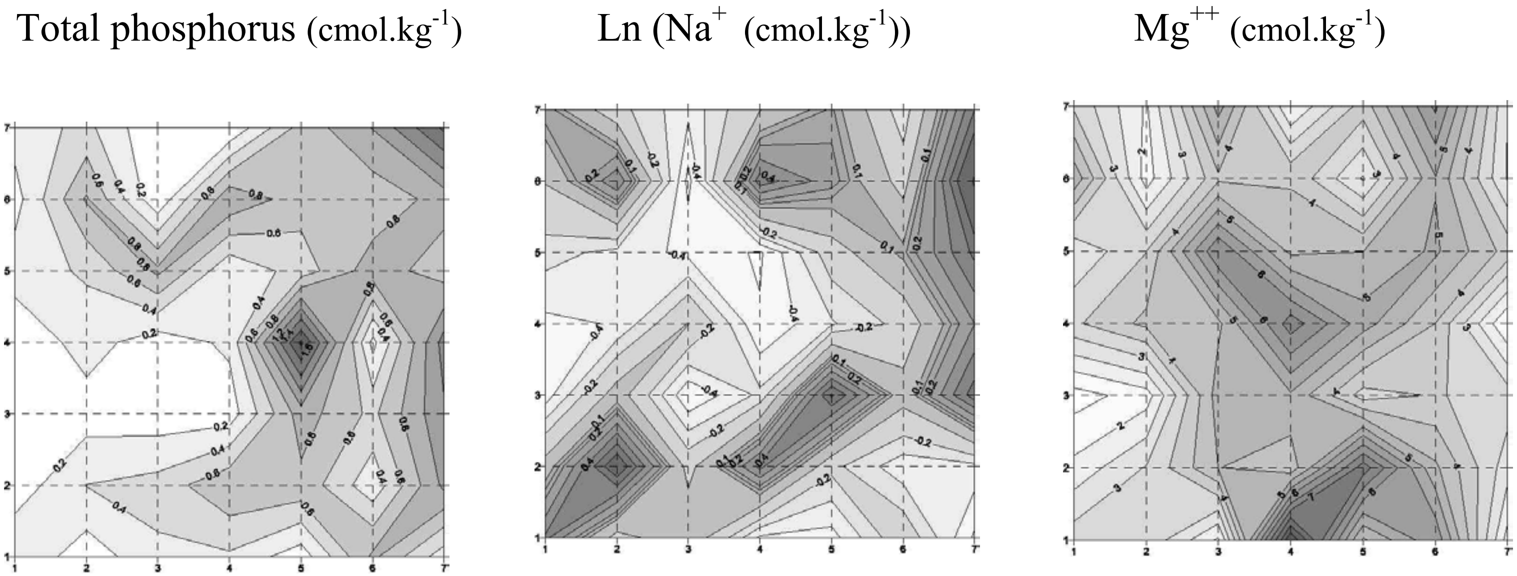


Ca^{++} (cmol.kg^{-1})

Mg^{++} (cmol.kg^{-1})

Figure 4.5 Kriged contour maps of soil chemistry variables that had an autocorrelated phase in the 15-year-old forest. One unit on the grid is equivalent to a distance of 5 m.

45-year-old forest



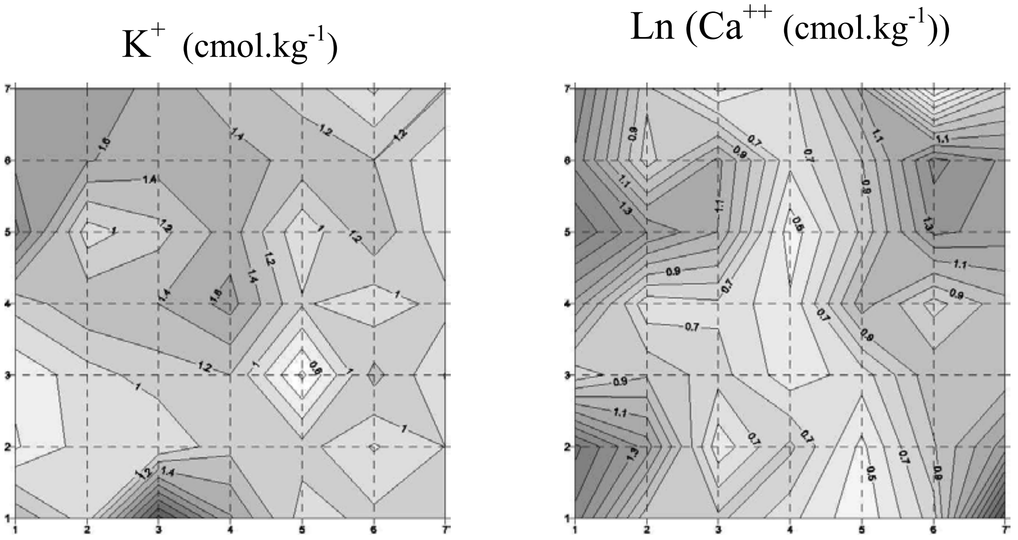
Total phosphorus (cmol.kg^{-1}) Ln (Na^{+} (cmol.kg^{-1})) Mg^{++} (cmol.kg^{-1})

Figure 4.6 Kriged contour maps of soil chemistry variables that had an autocorrelated phase in the 45-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

grid delimited by the 1 and 4 y co-ordinate lines (bottom half of the map in figure 4.5) and several of their gaps coincided in location. The spatial pattern of total carbon was nearly complementary to that of phosphorus, its concentration was highest in the areas where phosphorus was scarcer.

In the 45-year-old forest those soil chemistry components that were autocorrelated (total phosphorus, sodium and magnesium ions; table 4.2) had very localised areas of high concentration forming a few small patches (figure 4.6). The position of these patches for different variables did not coincide. In the 75-year-old forest only potassium and calcium ions were autocorrelated (table 4.3) and their spatial patterns differed (figure 4.7). In both cases there was a single grid intersection that had a very high concentration ((3,1) for potassium and (7,1) for calcium). In the 100-year-old forest total phosphorus and sodium, calcium and magnesium ions were

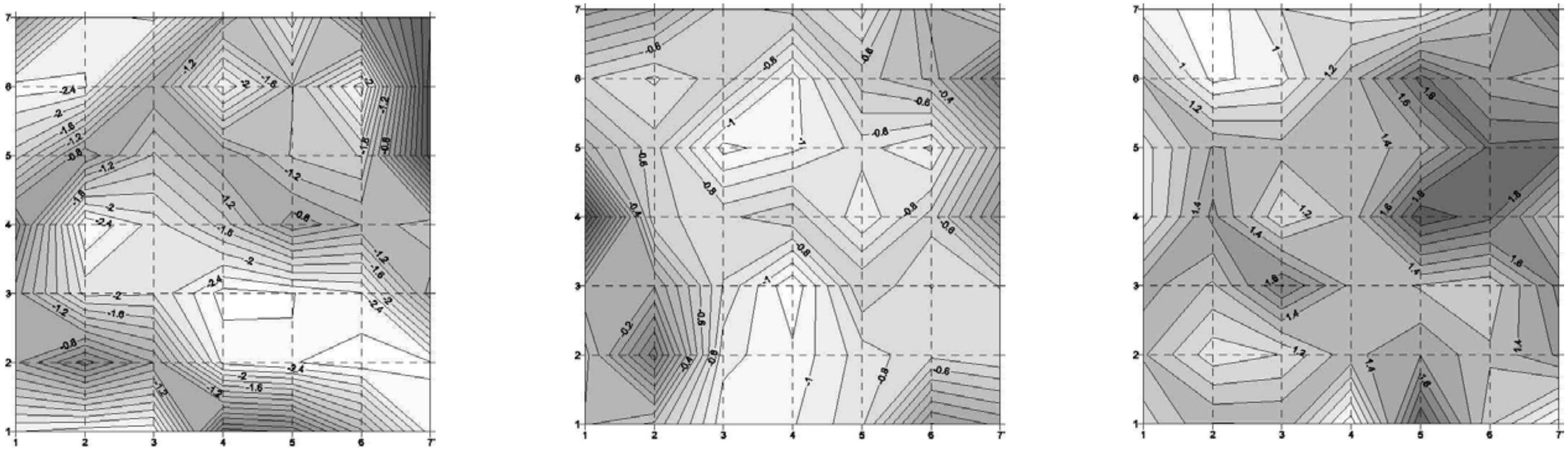
75-year-old forest


K^+ (cmol.kg^{-1})

Ln (Ca^{++} (cmol.kg^{-1}))

Figure 4.7 Kriged contour maps of soil chemistry variables that had an autocorrelated phase in the 75-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

100-year-old forest



Ln (total phosphorus (cmol.kg^{-1}))   Ln (Na^{+} (cmol.kg^{-1}))   Ln (Ca^{++} (cmol.kg^{-1}))

Ln (Mg^{++} (cmol.kg^{-1}))

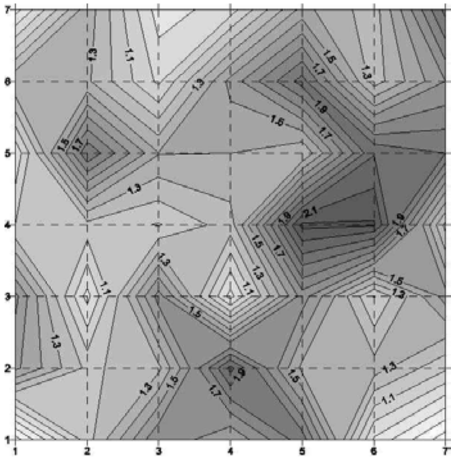


Figure 4.8. Kriged contour maps of soil chemistry variables that had an autocorrelated phase in the 100-year-old forest. The name of those variables that have been log-transformed to meet distributional assumptions are preceded by Ln. One unit on the grid is equivalent to a distance of 5 m.

autocorrelated (table 4.3). In general, these elements had a relatively uniform concentration across the grid, except for a few very localised areas of high concentration (figure 4.8). In the case of total phosphorus there was also a noticeable gap between the co-ordinates (4,3) and (7,1). Magnesium and calcium ions displayed very similar spatial patterns with the largest patch of high concentration located between (5,3) and (7,6).

Spatial patterns of the macroinvertebrate community in different successional stages

Shannon's Diversity Index

Considering only the values at each intersection, the 15-year-old forest grid had the largest number of intersections with low macroinvertebrate diversity values both in litter and soil (figure 4.9). The frequency of intersections with low diversity values decreased with the age of the forest, reaching a more uniformly high diversity across the grid in the 100-year-old forest (figure 4.9). However, Shannon's index of diversity was only autocorrelated at the 5 m-grain interval in the soil and litter of the 100-year-old successional stage (table 4.3). In the other successional stages independence between observations was reached at a smaller distance. Shannon's index in the 100-year-old forest in general had a uniform value above 1.8 except for three patches in the litter (centred at (3,1), (7,6) and (1,6)) where diversity dropped below 0.8, and three patches in the soil (centred at (3,4), (4,6) and (5,5)) where diversity dropped below 1.6 (figure 4.10).

Individual macroinvertebrate taxa

Seven macroinvertebrate taxa were considered dominant (table 4.4) and all of them showed a significant patchy (aggregated (I_a) or crowded (J_a)) distribution in at least one successional stage (table 4.5). However, none of them was significantly patchy across all successional stages. Chilopoda and Diplopoda were the taxa that had the highest frequency of patchiness, being significantly aggregated or crowded in the soil of three successional stages (table 4.5).

	15-year-old			45-year-old			75-year-old			100-year-old		
	mean	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**	**mean**	**SD**	**CV**
Litter												
Chilopoda	0.75	1.15	1.53	1.37	1.53	1.12	0.97	1.08	1.11	1.11	1.33	1.20
Diplopoda	0.90	1.64	1.82	2.21	2.40	1.09	2.21	2.40	1.09	3.62	3.84	1.06
Coleoptera	0.95	1.31	1.38	4.13	3.30	0.80	5.48	4.38	0.80	4.22	3.04	0.72
Formicidae	1.54	5.73	3.72	3.33	13.86	4.16	1.75	4.42	2.53	3.10	13.49	4.35
Diplura	0.08	0.33	4.13	0.43	0.86	2.00	0.81	1.59	1.96	0.52	1.26	2.42
Coleoptera larvae	1.10	1.34	1.22	1.95	1.87	0.96	3.00	2.31	0.77	2.16	2.50	1.16
Other larvae and Pupae	0.46	2.06	4.48	0.62	2.11	3.40	0.86	2.63	3.06	2.71	11.08	4.09
Shannon's H'	1.18	0.71	0.60	1.64	0.49	0.30	1.80	0.41	0.23	1.75	0.52	0.30
Soil												
Chilopoda	1.44	1.59	1.10	2.67	2.40	0.90	3.97	4.52	1.14	4.33	3.23	0.75
Diplopoda	1.14	1.45	1.27	1.59	2.25	1.42	2.68	3.24	1.21	5.10	4.13	0.81
Coleoptera	1.87	1.72	0.92	2.22	2.53	1.14	5.40	3.62	0.67	6.44	4.83	0.75
Formicidae	3.48	13.95	4.01	2.90	6.82	2.35	6.59	9.85	1.49	6.33	7.58	1.20
Diplura	0.60	1.01	1.68	1.32	1.43	1.08	1.63	2.03	1.25	2.62	2.92	1.11
Coleoptera larvae	1.67	2.50	1.50	2.59	2.45	0.95	4.33	2.87	0.66	4.41	3.68	0.83
Other larvae and Pupae	1.13	3.07	2.72	1.30	4.45	3.42	2.44	3.26	1.34	2.60	4.07	1.57
Shannon's H'	1.46	0.51	0.35	1.59	0.47	0.30	1.89	0.33	0.17	1.96	0.27	0.14

Table 4.4 Mean abundances, standard deviations (S.D) and coefficients of variation (CV) of macroinvertebrate taxa and Shannon's diversity index . Data for the litter and soil community in different successional stages is presented. Includes the counts for all 49 grid intersections in each successional stage.

	15-year-old				45-year-old				75-year-old				100-year-old			
	Ia	**P_{Ia}**	**Ja**	**P_{Ja}**	**Ia**	**P_{Ia}**	**Ja**	**P_{Ja}**	**Ia**	**P_{Ia}**	**Ja**	**P_{Ja}**	**Ia**	**P_{Ia}**	**Ja**	**P_{Ja}**
LITTER																
Chilopoda	0.972	0.490	1.002	0.495	1.209	0.113	0.958	0.724	0.890	0.745	0.967	0.707	1.180	0.207	1.087	0.138
Diplopoda	0.970	0.504	0.956	0.634	0.942	0.586	0.969	0.762	0.903	0.694	1.007	0.460	1.391	**0.022**	0.159	1.050
Coleoptera	0.845	0.872	0.966	0.681	1.230	0.093	1.024	0.304	0.994	0.436	1.008	0.441	1.608	**0.002**	1.102	**0.018**
Formicidae	0.877	0.760	0.860	0.721	0.916	0.659	1.020	0.508	0.863	0.772	1.111	0.277	1.045	0.325	1.210	**0.018**
Diplurans	1.174	0.147	1.158	0.396	1.080	0.267	0.877	0.871	0.963	0.522	0.971	0.580	1.000	0.438	1.480	**0.005**
Coleop. larv.	1.232	0.084	1.102	0.119	1.944	**<0.001**	1.047	0.204	1.107	0.223	0.988	0.592	1.321	**0.040**	1.119	0.057
Other larv. and Pupae	0.976	0.520	1.304	0.158	0.976	0.514	1.265	0.132	0.870	0.752	1.115	0.318	1.016	0.378	1.190	**0.047**
SOIL																
Chilopoda	1.455	**0.013**	1.034	0.290	0.987	0.458	0.900	0.984	1.649	**0.002**	1.003	0.462	1.090	0.393	1.120	**0.001**
Diplopoda	1.390	**0.032**	0.994	0.511	1.064	0.306	1.170	**0.015**	1.115	0.226	0.982	0.590	1.824	**<0.001**	1.066	**0.052**
Coleoptera	0.985	0.465	0.903	0.986	1.404	**0.025**	0.950	0.817	0.831	0.863	0.970	0.782	1.510	**0.007**	1.090	**0.012**
Formicidae	0.948	0.579	1.211	0.189	1.252	0.079	0.923	0.767	1.219	0.103	1.170	**0.030**	1.006	0.404	1.210	**0.007**
Diplura	1.120	0.199	0.991	0.537	0.955	0.554	1.068	0.150	0.768	0.963	0.982	0.603	1.074	0.277	1.046	0.187
Coleop. larv.	1.085	0.266	1.196	**0.028**	1.193	0.114	0.981	0.652	0.965	0.511	1.018	0.296	2.238	**<0.001**	1.104	**0.009**
Other larv. and Pupae	1.061	0.340	1.370	0.060	1.224	0.090	0.782	0.925	0.947	0.572	0.969	0.163	1.243	0.096	1.043	0.310

Table 4.5. SADIE indices for macroinvertebrate taxa in the soil and litter of different successional stages. The Index of Aggregation (Ia) and Index of Clustering (Ja) are presented with P-values for randomisation significance tests ($P \leq 0.05$ in bold).

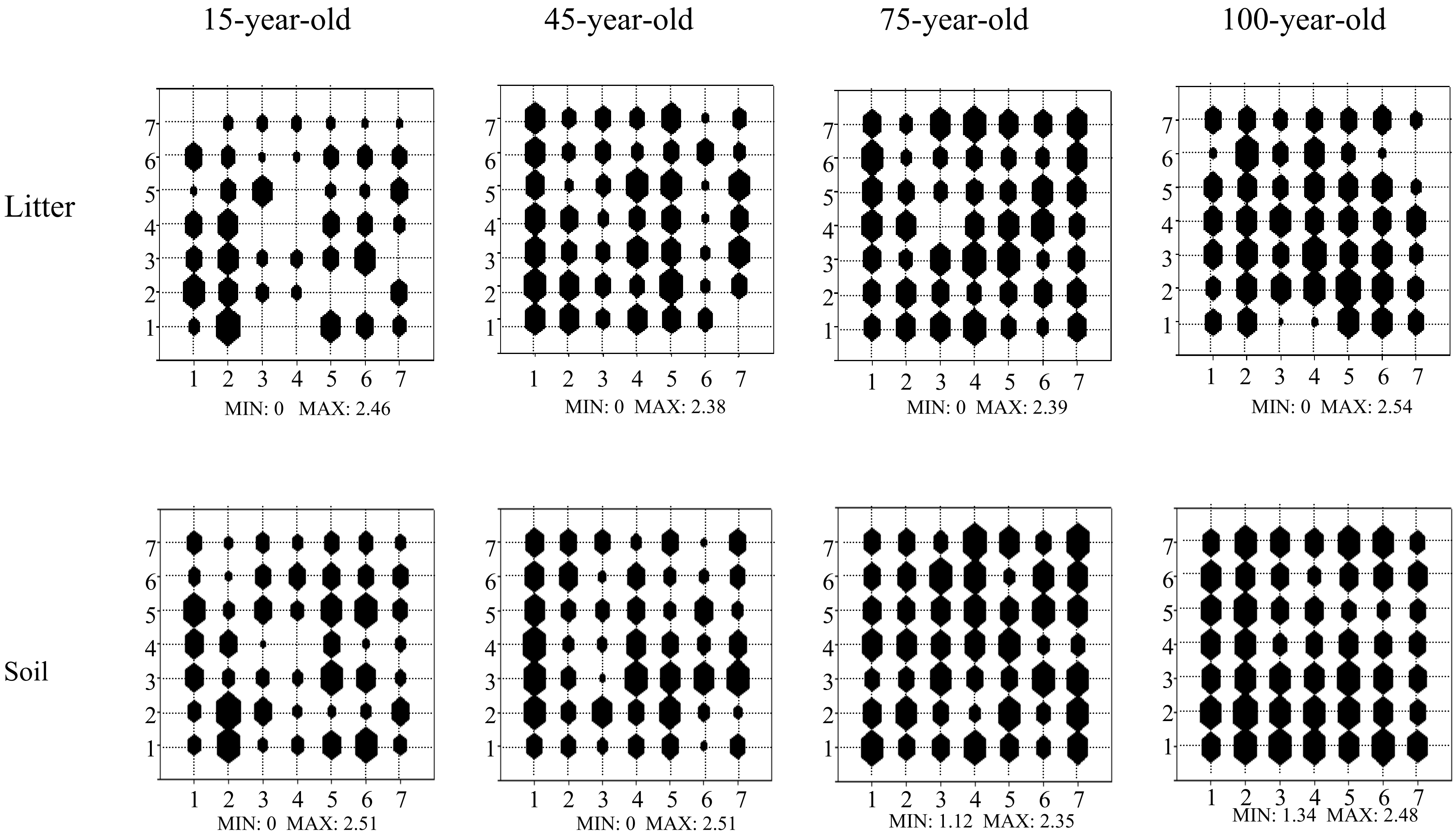
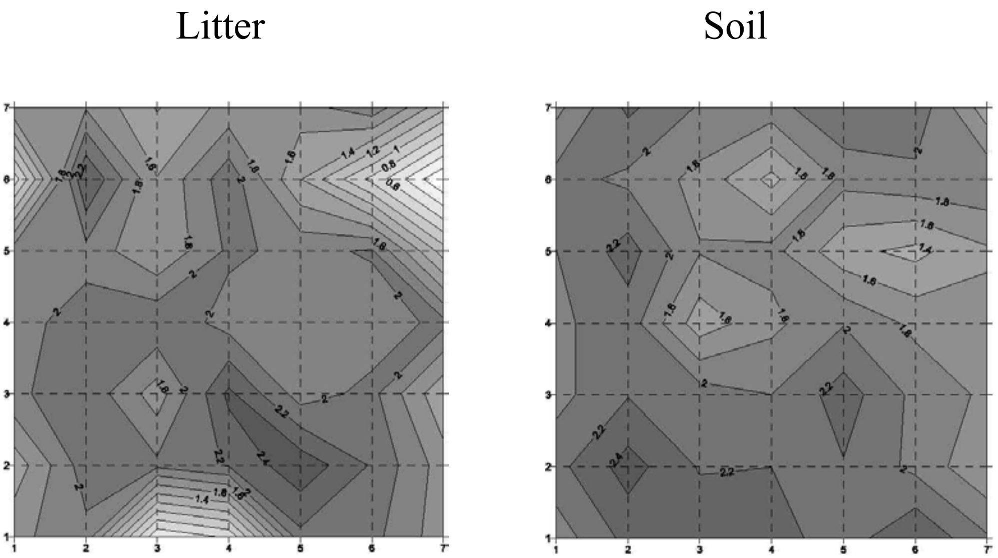


Figure 4.9. Bubble maps of Shannon's index of diversity (H') of macroinvertebrate taxa in the grids of different successional stages. The size of each symbol is proportional to the value of H' at that intersection. One unit on the grid is equivalent to a distance of 5m. Minimum and maximum values for each grid are also presented.

Amongst all successional stages, the 100-year-old forest showed the highest frequency of patchiness of macroinvertebrate taxa, both in soil and litter. Furthermore, in the litter patchiness was only recorded in the 100-year-old forest, except for the larvae of Coleoptera that were also significantly aggregated in the litter of the 45-year-old forest (table 4.5).

100-year-old forest



Litter

Soil

Figure 4.10. Kriged contour maps of Shannon's diversity index (H') of macroinvertebrate taxa in the litter and soil of the 100-year-old forest. One unit on the grid is equivalent to a distance of 5 m.

In the soil of the 15-year-old forest, the distributions both of Chilopoda and Diplopoda were significantly more clustered (46% and 36%) and gapped (50% and 53%) than expected at random (figure 4.11). Coleoptera larvae were congregated in a single patch (J_a=1.19, P=0.028) centred on the co-ordinate (3,3) (figure 4.11).

In the litter of the 45-year-old forest only Coleoptera larvae displayed a significantly patchy distribution. The distribution of this larval group was 61% more clustered and 79% more gapped than expected at random. One major aggregation was centred in (2,2) and most of the grid was covered by significant gaps (figure 4.12). In the soil of

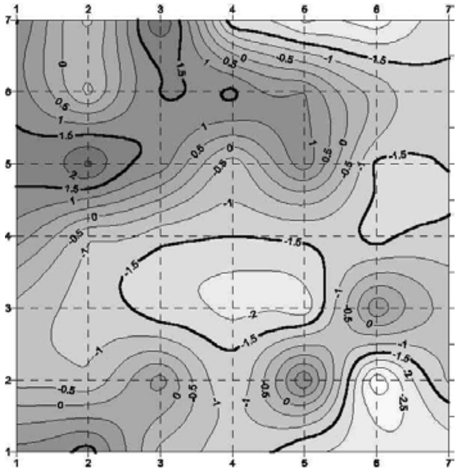
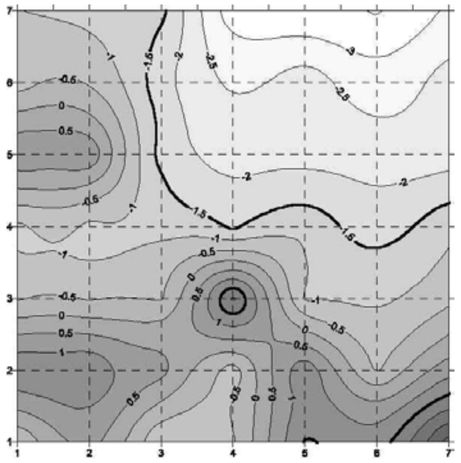
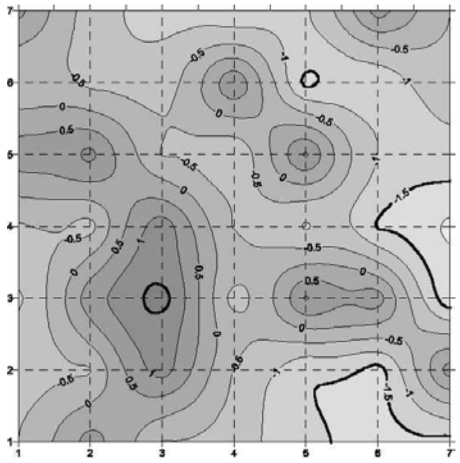
15-year-old forest	Chilopoda	Diplopoda	Coleoptera larvae
Litter	N.S.A.	N.S.A.	N.S.A.
Soil			

Figure 4.11 Contour maps of υ values for those dominant macroinvertebrate taxa that displayed significant patchiness (P-values for Ia or Ja <0.05) in the litter or soil of the 15-year-old forest. Light areas represent low υ values and dark areas high υ values. Dark contours enclose areas where there is more than 50% aggregation ($\upsilon \geq 1.5$) or emptiness ($\upsilon \leq -1.5$) than expected at random. Mean values ($\bar{\upsilon}i$ and $\bar{\upsilon}j$) are presented with overall P-values for randomisation significance tests. N.S.A. stands for not significantly aggregated (P-values for Ia and Ja >0.05). One unit on the grid is equivalent to a distance of 5 m.

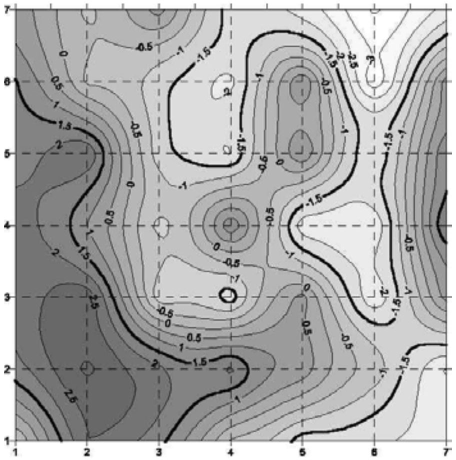
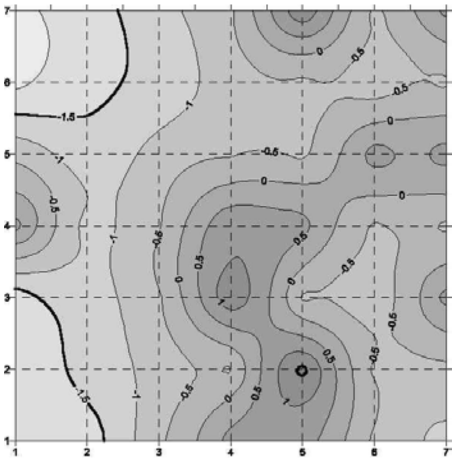
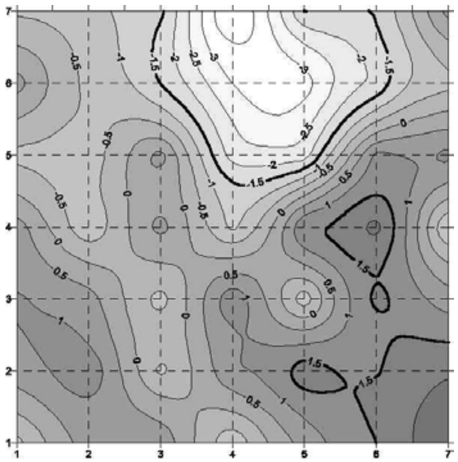
45-year-old forest	Diplopoda	Coleoptera	Coleoptera larvae
Litter	N.S.A.	N.S.A.	 $\bar{\upsilon}_i$=1.609 (P=0.0015); $\bar{\upsilon}_j$=-1.791
Soil	 $\bar{\upsilon}_i$=0.906 (P=0.682); $\bar{\upsilon}_j$= -1.011	 $\bar{\upsilon}_i$=1.176 (P=0.117); $\bar{\upsilon}_j$= -1.396	N.S.A.

Figure 4.12. Contour maps of υ values for those dominant macroinvertebrate taxa that displayed significant patchiness (P-values for Ia or Ja <0.05) in the litter or soil of the 45-year-old forest. Light areas represent low υ values and dark areas high υ values. Dark contours enclose areas where there is more than 50% aggregation (υ≥1.5) or emptiness (υ≤-1.5) than expected at random. Mean values ($\bar{\upsilon}_i$ and $\bar{\upsilon}_j$) are presented with overall P-values for randomisation significance tests. N.S.A. stands for not significantly aggregated (P-values for Ia and Ja >0.05). One unit on the grid is equivalent to a distance of 5 m.

the 45-year-old forest. Diplopoda were crowded in a single small patch (J_a=1.17, P=0.015) centred on (5,2) and the distribution of Coleoptera was not significantly clustered, but was 39% more gapped than expected at random. A single significant gap in the distribution of Coleoptera was centred on the co-ordinate (4,7).

Chilopoda and Formicidae had a significantly patchy distribution in the soil of the 75-year-old successional stage (figure 4.13). The distribution of Chilopoda was 58% more clustered and 40% more gapped than expected at random. Most of the aggregation of this taxon was found in a cluster centred on (7,7) and a major part of the sampling grid was covered with significant gap areas. Formicidae were crowded in a single patch (J_a=1.17, P=0.030) centred in (4,4).

The spatial distribution of all seven common macroinvertebrate taxa was patchy either in the soil or litter of the 100-year-old forest. Many taxa were patchy both in the litter and in the soil, however Chilopoda had a patchy distribution only in the soil and Diplura and other larvae and pupae only in the litter (figure 4.14). In the soil Chilopoda were crowded in a single patch (J_a=1.12, P<0.001) centred between (3,3) and (4,3). Around that patch the abundance of Chilopoda gradually decreased towards the edges of the grid. The distribution of Diplopoda in the litter was not clustered but it was 37% more gapped than expected at random. There were three significant gaps in the grid, the most extensive being centred between (1,4) and (4,1). In the soil, this taxon was 56% more clustered and 67% more gapped than expected from random. The most extensive cluster was centred in (6,2) and there was a considerable area covered by a gap centred in (3,7). In the litter the distribution of Coleoptera was 46% more clustered and 60% more gapped than expected from random. The most extensive cluster was centred on two points (3,3 and 5,2). The only significant gap was centred on (2,7). In the soil, their distribution was 46% more clustered and 39% more gapped than expected at random. There was an extensive horseshoe-shaped cluster surrounding the co-ordinate (5,2) and a major gap centred between (1,2) and (1,3).

75-year-old forest

Chilopoda

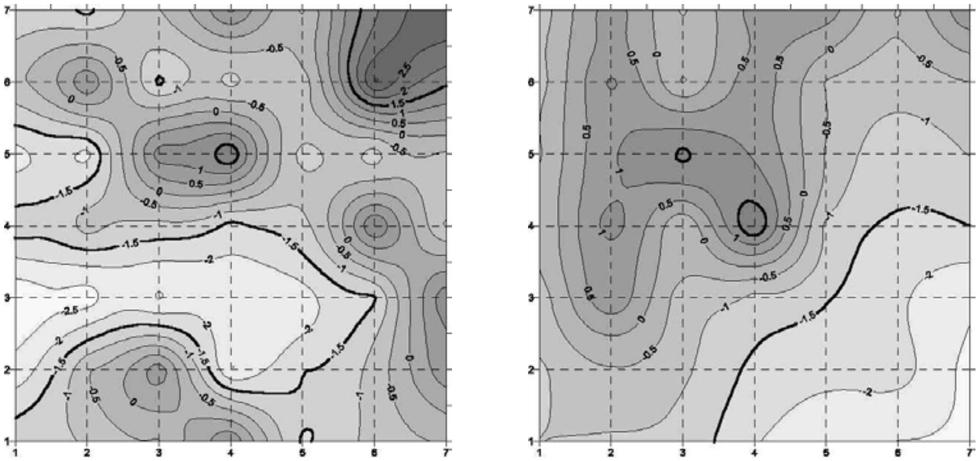
Formicidae

Litter

N.S.A.

N.S.A.

Soil



$\bar{\upsilon}_i$=1.578 (P=0.0026); $\bar{\upsilon}_j$=-1.398

$\bar{\upsilon}_i$=1.070 (P=0.297); $\bar{\upsilon}_j$=-1.221

Figure 4.13. Contour maps of υ values for those dominant macroinvertebrate taxa that displayed significant patchiness (P-values for Ia or Ja <0.05) in the litter or soil of the 75-year-old forest. Light areas represent low υ values and dark areas high υ values. Dark contours enclose areas where there is more than 50% aggregation (υ≥1.5) or emptiness (υ≥-1.5) than expected at random. Mean values ($\bar{\upsilon}$i and $\bar{\upsilon}$j) are presented with overall P-values for randomisation significance tests. N.S.A. stands for not significantly aggregated (P-values for Ia and Ja >0.05). One unit on the grid is equivalent to a distance of 5 m.

100-year-old forest

Chilopoda

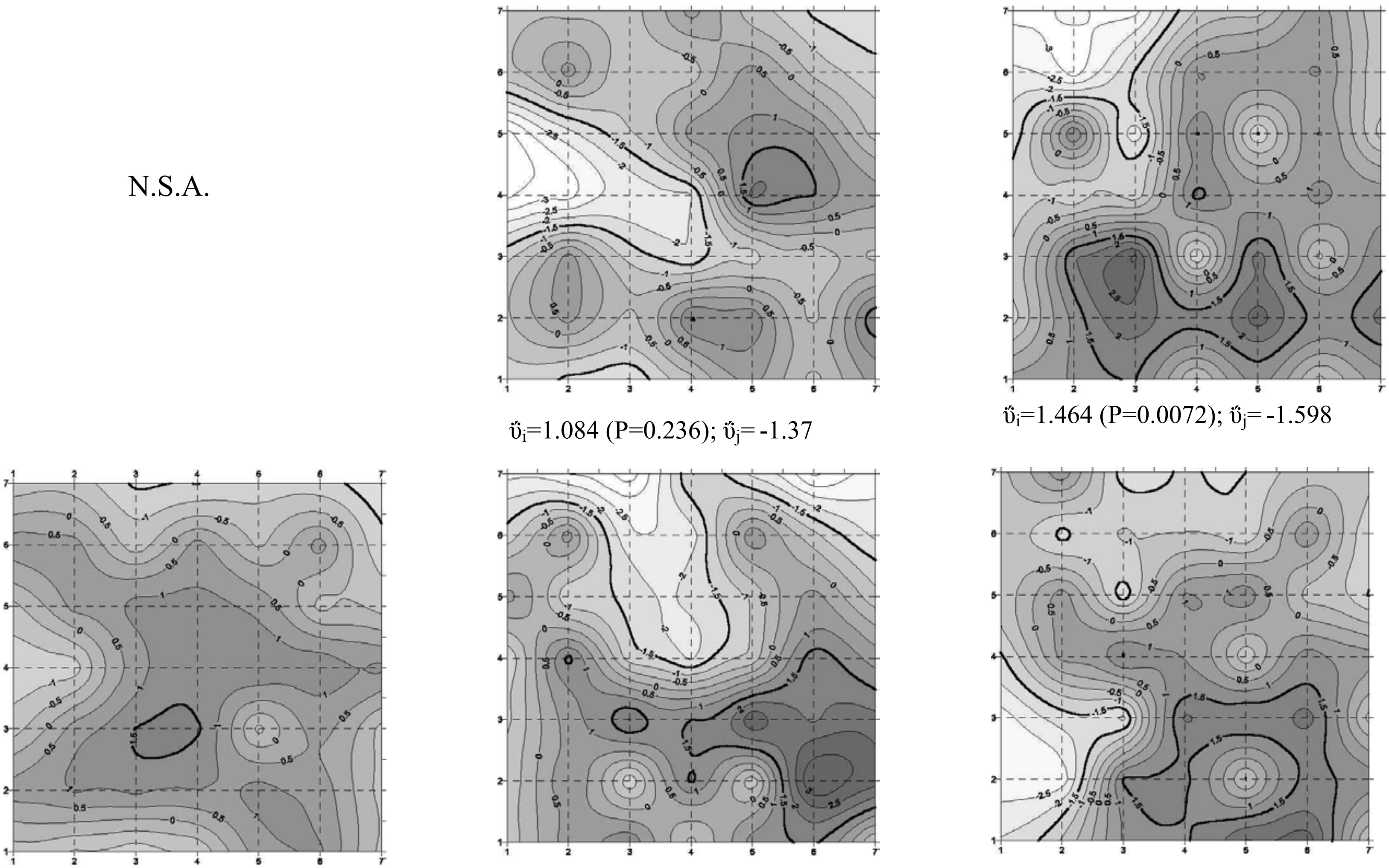
Diplopoda

Coleoptera

Litter

N.S.A.

Soil



$\bar{\upsilon}_i=1.084$ ($P=0.236$); $\bar{\upsilon}_j=-1.37$

$\bar{\upsilon}_i=1.464$ ($P=0.0072$); $\bar{\upsilon}_j=-1.598$

$\bar{\upsilon}_i=1.011$ ($P=0.408$); $\bar{\upsilon}_j=-1.019$

$\bar{\upsilon}_i=1.559$ ($P=0.004$); $\bar{\upsilon}_j=-1.678$

$\bar{\upsilon}_i=1.462$ ($P=0.009$); $\bar{\upsilon}_j=-1.393$

Figure 4.14 Contour maps of υ values for those dominant macroinvertebrate taxa that displayed significant patchiness (P-values for Ia or Ja <0.05) in the litter or soil of the 100-year-old forest. Light areas represent low υ values and dark areas high υ values. Dark contours enclose areas where there is more than 50% aggregation ($\upsilon \geq 1.5$) or emptiness ($\upsilon \geq -1.5$) than expected at random. Mean values ($\bar{\upsilon}_i$ and $\bar{\upsilon}_j$) are presented with overall P-values for randomisation significance tests. N.S.A. stands for not significantly aggregated (P-values for Ia and Ja >0.05). One unit on the grid is equivalent to 5 m.

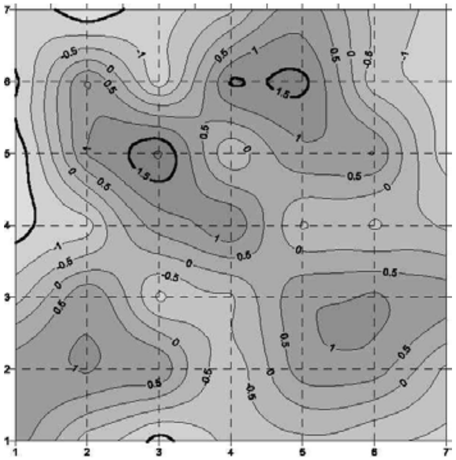
100-year-old forest

Formicidae

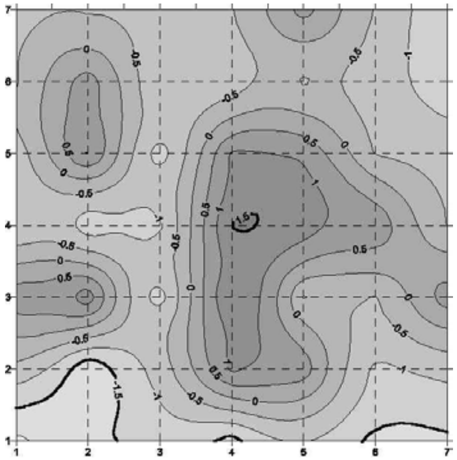
Diplura

Coleoptera larvae

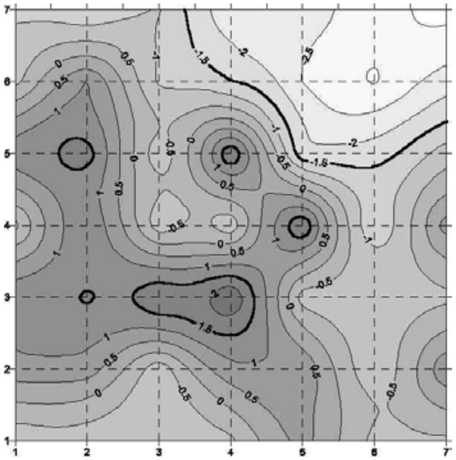
Litter



$\ddot{\upsilon}_i=1.089$ (P=0.300); $\ddot{\upsilon}_j=-1.048$

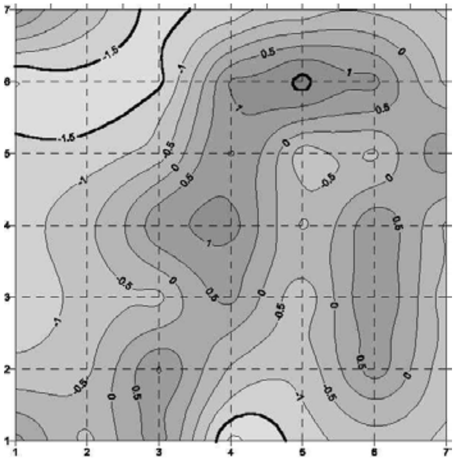


$\ddot{\upsilon}_i=1.026$ (P=0.366); $\ddot{\upsilon}_j=-$



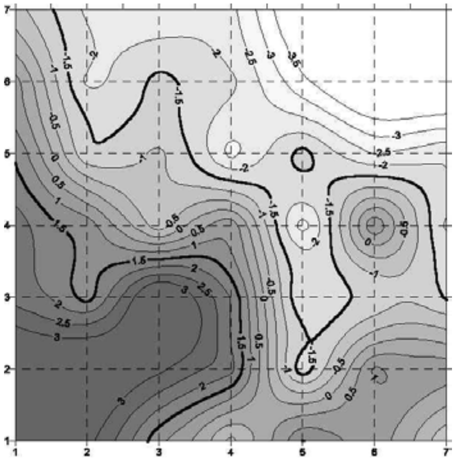
$\ddot{\upsilon}_i=1.324$ (P=0.034); $\ddot{\upsilon}_j=-1.323$

SOIL



$\ddot{\upsilon}_i=0.985$ (P=0.452); $\ddot{\upsilon}_j=-1.000$

N.S.A.



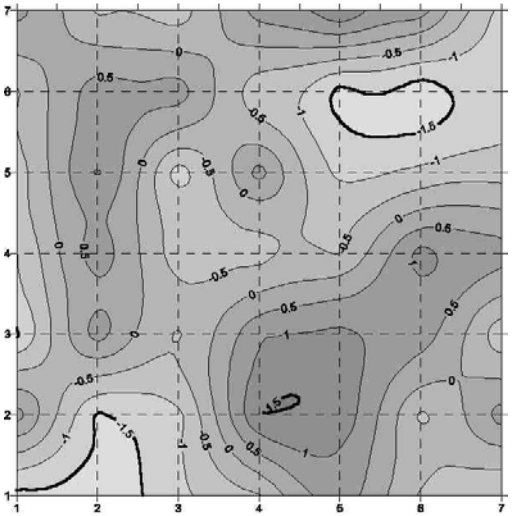
$\ddot{\upsilon}_i=2.082$ (P<0.001); $\ddot{\upsilon}_j=-2.274$

Figure 4.14 continuation...

100-year-old forest

Other larvae and pupae

Litter



$\tilde{\upsilon}_i$=0.906 (P=0.119); $\tilde{\upsilon}_j$= -1.012

Soil

N.S.A.

Figure 4.14 continuation...(ends)

Formicidae were crowded in a single patch both in the litter (J_a=1.21, P=0.018) and in the soil of the 100-year-old forest (J_a=1.090, P=0.007; figure 4.14). In the litter the centre of the patch is difficult to determine because there were three points where υ_i>1.5 ((3,5), (4,6) and (5,6)). In the soil the centre of the patch was located on (5,6). Diplurans in the litter were also crowded in a single patch (J_a=1.48, P=0.005) centred on (4,4). In the litter the distribution of Coleoptera larvae was 32% more clustered and 32% more gapped than expected at random. There were five small clusters arranged in a horseshoe-shape around the (3,4) co-ordinate, and a single extensive gap centred on the (7,7) corner of the grid. The distribution of Coleoptera larvae in the soil was the only distribution that clearly followed a gradient pattern rather than a set of clusters. This taxon was most abundant in the (1,1) corner and gradually decreased diagonally in abundance moving away from this point, ending in a major gap area in the (7,7) corner. As a result of this pattern, clustering analysis determined that the distribution was 108% more clustered and 127% more gapped than expected at random. In the litter, other larvae and pupae were crowded (J_a=1.190, P=0.047) in a small patch centred on (4,2).

The patterns of aggregation of some macroinvertebrate taxa and the spatial distribution of resources and soil chemical characteristics seemed to have matching patterns in the kriged maps. In the 15-year-old forest the areas of high abundance of Chilopoda in the soil coincided with areas of high concentration of carbon and with the general pattern of abundance of pine needles. The distribution of Diplopoda in the soil of the same grid was similar to the spatial pattern of the concentration of total carbon except for the gap surrounding the (7,7) corner (figures 4.1, 4.5 and 4.11).

In the soil of the 45-year-old forest, the centres of highest aggregation and the location of most gaps in the spatial distribution of Coleoptera coincided with the areas of highest and lowest abundance of woody and reproductive material (figures 4.2 and 4.12). In the litter of this same grid, the area of highest aggregation and the location of gaps in the distribution of Coleoptera larvae matched the areas of highest and lowest abundance of pine needles (figure 4.2 and 4.12).

In the 75-year-old forest no correspondence was found between the location of patches and gaps in the distribution of Chilopoda and Formicidae and any of the litter components or soil chemistry variables mapped (figures 4.3 and 4.13). In the litter and soil of the 100-year-old forest the gradient of increasing abundance of *Quercus* leaves from the (1,1) corner to the (7,7) corner of the grid coincided with the pattern of increasing abundance of Coleoptera larvae in the same direction (figures 4.4 and 4.14).

Discussion

Microenvironment

An increasing tree taxonomic diversity is often accompanied by an increase in the canopy cover heterogeneity (Pacala & Deutschman, 1995). Given the increase in tree taxonomic diversity with forest age in Tarantulas, an increase the light penetration heterogeneity within-plots should also develop. The heterogeneity in the light environment then promotes high variability in microenvironmental conditions in the soil. The canopy cover, soil temperature and soil water content did not vary by more than 21% (CV <0.21) within any of the successional plots. Furthermore, this variability did not increase through succession, suggesting that at the within-plot scale the increasing heterogeneity in the tree community does not drive heterogeneity in the soil microenvironment.

Similar results were found in three successional stages (Soybean field and *c.* 28- and 70-year-old) of Hardwood growth in Michigan (Gross *et al.*, 1995). Very low within-plot variability in soil water content (10-29%) was found when sampling at 2.5 m grain intervals. Using smaller grain intervals (2 m, 20 cm and 10 cm) Farley & Fitter (1999) also found little within-plot variation in soil water content in an old mixed woodland in North Yorkshire. In contrast, Robertson *et al.* (1988) reported greater variability (59%) in an early successional vegetation in Southeast Michigan at the 1-10 m scale. However, results presented here support those of the three studies mentioned above, in that within-plot heterogeneity was greater in soil nutrient concentration than in soil water content. The explanation may lie in the fact that vegetation diversity more strongly influences the spatial distribution of soil water resources when these are very limited. Stratton & Meinzer (2000) showed that different tree species in a very diverse Hawaiian dry forest used water resources at different depths in the soil, and therefore patchiness in soil water resources was expressed in a vertical spatial dimension at the within-plot scale.

The small variation in microenvironmental conditions with-in plots found in Tarantulas suggests that the changes in canopy diversity through 100 years of succession do not translate into pronounced microenvironment heterogeneity that could strongly affect the soil biogeochemistry. However, Pacala & Deutschman (1995) proposed that the heterogeneous microenvironment created by a diverse canopy is a determinant of productivity and organic matter cycling in the longer term (between 100 and 2000 years of succession). They compared the outcome of a mean-based (spatially uniform) version of the forest model SORTIE with a similar version that included spatially explicit variation in canopy structure and therefore microenvironment around the mean. They found that the mean model could only account for 50% of the primary productivity in Northeastern hard-woods in North America while the spatially-heterogeneous model was able to predict all of the productivity. They predicted by extension that horizontally uniform forest would store 50% less below-ground biomass and 50% less un-decomposed organic matter than a heterogeneous forest.

In El Rincón, at the landscape scale (hundreds of metres to hectares), the spatial patterning in soil microenvironmental variables may be determined by the vegetation mosaic created by unsynchronised disturbance. In Chapter 3 some differences in the mean temperature and canopy cover were found between plots, which may result in patchiness higher in the heterogeneity hierarchy.

Litter components and soil nutrient concentrations

Contrary to microenviromental conditions, all of the litter component and nutrient concentration variables tested had more than 25% within-plot variation and significant spatial structures in at least one of the successional stages. For most variables that were spatially structured within the plots, the autocorrelation continued beyond 24 m (the maximum distance tested). This was evident in the fact that a power equation was frequently used, nested within an exponential equation to model the variogram and was reflected in the continuous increase in the semivariance values at all separation distances. In short, the spatial independence of observations was not attained within the study's range. However, most variograms showed a tendency to level-off which indicates that the study's range covered the most distinct area of influence of individual patches. The remaining spatial autocorrelation may be caused by spatial structures operating simultaneously, at a greater scale, higher in the heterogeneity hierarchy. In the few cases were the variogram did not show a tendency to level-off, the spatial structures being detected were either unidirectional gradients or large patches of which the majority of area of extent was not included in the study's range. Obviously, for those variables that were not autocorrelated, their spatial structures operated at a radically different scale, either smaller or larger.

It was predicted that as the tree diversity increased, the distribution of litter components would become more patchy because neighbouring trees from different species would develop contrasting zones of influence in the characteristics of the litter. Although all litter components had higher variability than 25% in the four successional stages, the expected increase in within-plot variance with forest age was only observed in the abundance of *Pinus* and *Quercus* litter. These genera dominate early-mid succession and their dominance decreases in late succession (see Chapter 3, figure 3.2). It is expected that the abundance of their litter becomes more patchy with succession as their dominance declines and therefore the within-plot variance increases. In contrast, the abundance of Lauraceae leaves followed the opposite trend, their variance diminished with succession. This was probably because in these forests it is a late-successional family. In early-mid succession very few individuals are present and they are only small in stature. The abundance of their litter was so low in the 15- and 45-year-old forests that no geostatistical analysis was possible. In the 75-year-old forest, the abundance of their litter was high enough to perform variography, but no significant autocorrelation was detected, probably as a result of the patch size being smaller than the scale studied. In late succession, Lauraceae trees become more abundant and robust, and therefore the patches of leaves created by their canopy is expected to increase in size. In the 100-year-old forest the Lauraceae litter formed spatial structures detectable at the within-plot scale.

If more tree species coexist per hectare in late succession, it is likely that more litter components will become spatially structured at a scale influenced by single trees. This is because tree species may have different influences on the components of the litter. For example, one tree species may produce more abundant leaf litter, while another may loose more twigs or heavier fruits. Therefore, the more species coexisting, the more likely it would be to find patches with distinct proportions of different litter components. Three pieces of evidence are consistent with this hypothesis. First, in early-mid succession more often than in late succession a power model was required as the first component of the nested variogram model. This is an indication of the predominance in early successional variograms of a sustained increase in semi-variance. Therefore in early succession, larger-scale patchiness dominated the autocorrelation signal. In late succession, where power models were rarely required as the first variogram structure, the patchy distribution of litter components within the plot size were more influential.

The second piece of evidence comes from considering only the litter components that were present in all successional stages (total litter mass, unidentifiable material, woody and reproductive parts and other species). In the 15-year-old forest none of these were significantly autocorrelated, while in the 100-year-old all of them were. Thirdly, the variogram of pine needle abundance (the only identified foliar species that was present in this successional stage) did not have a tendency to level-off, suggesting that, the patches of needles in this successional stage are larger than the range of the study. In summary, results are consistent with the hypothesis that in early succession where the tree community is more homogeneous, the scale at which litter components are structured is larger than in late succession.

For litter variables, it was hypothesised that the more diverse the tree community, the more patchy the distribution of nutrients in the top soil would become. This is true for the top soil in particular because, in terms of nutrients availability and cycling, the surface soil seems to be where most plant-soil biochemical interaction concentrates in forests (Gross *et al.*, 1995). Fine root growth, mortality and density, which are usually associated with nutrient uptake, occur predominantly in the upper 20 cm of forest soils (Hendrick & Pregitzer, 1996). If each tree species generates a particular soil environment under its canopy, then under a diverse tree community soil chemical properties will be spatially very heterogeneous. The mechanisms by which tree species can influence the spatial distribution of nutrients in the upper soil are discussed in depth in Chapter 6. For the present discussion it suffices to say that there are two main pathways by which a single tree can create a zone of influence around itself. First, litter from different tree species varies in quantity, nutrient quality and litter fall timing (See Chapter 3), which are determinant factors for the nutrient release to the soil (Swift *et al.*, 1979). Second, trees regulate the proliferation and life span of fine roots according to nutrient availability in different areas (Burton *et al.*, 2000) consuming, and competing with other plants for nutrients in a spatially heterogeneous manner (for a review on nutrient mediated plant interactions see Day *et al.*, 2003).

Several studies have found patchy distributions of nutrients in forests at the patch-scale, particularly different forms of available nitrogen (Farley & Fitter, 1999; Ehrenfeld *et al.*, 1997; Robertson *et al.*, 1988; Gross *et al.*, 1995), potassium (Lechowicz & Bell, 1991; Halaj & Wise, 2002; Jackson & Caldwell, 1993) and phosphate (Jackson & Caldwell, 1993; Farley & Fitter, 1999). Apart from carbon, all of the nutrients studied here desplayed CVs greater than 0.25 in all successional stages and often had patchy distributions in the plots. However, little support was found for the hypothesis that the distribution of nutrients became more heterogeneous with succession. Only total phosphorus concentration followed the hypothesised increase in within-plot variance through succession. The phosphorus results are consistent with those obtained by Gross *et al.* (1995) for nitrogen availability in south-western Michigan. Their *c.* 28-year-old forest had higher within-plot CVs for several measures of nitrogen availability than their *c.*70-year-old forest. Further, the scale at which these variables were autocorrelated in the younger forest was often larger (>10m) than in the older forest (2.5 -10m).

The second heterogeneity prediction was not supported either, because the number of nutrients with patchy distributions did not increase with the age of the forest. The 15-year-old forest and the 100-year-old forest had the highest number of nutrient variables with autocorrelated patterns. Therefore evidence suggests that the heterogeneity of most nutrients in the soil does not increase with succession. However, some evidence was found of an association between the distribution of the leaves of particular tree species and the spatial distribution of nutrients. In the 15-year-old forest, calcium and magnesium ions seemed to follow contrasting patterns to those of pine needle accumulation. Similarly, in the 100-year-old forest, phosphorus concentration followed a contrasting pattern to the abundance of oak leaves. Both of these relationships can be explained by low concentration of calcium and magnesium in pine litter and a low concentration of phosphorus in oak litter (Chapter 3, figure 3.6). This suggests that the spatial patterns in the distribution of the litter from the most dominant tree genera in Tarantulas may have an effect in the patchiness of nutrient concentration in the top soil. Jackson & Caldwell (1993) found spatial associations between the distribution of plant species and the patches of soil nutrients in a sagebrush-steppe in the USA. The spatial distributions of tussock grasses and the patches of phosphate and potassium coincided in a 10 m × 12 m grid (sampling with a 1 m grain interval). In contrast the maps of the abundance of these nutrients did not match the distribution of the coexisting sagebrush plants. However, Ehrenfeld *et al.* (1997) did not find such a correlation between plants and nutrients in the New Jersey Pinelands. They found that little variation in extractable ammonia concentration and nitrogen mineralisation rates could be explained by the location of different tree species along transects. They suggested that any relationship may have been over-shadowed by the high temporal variability of nutrient availability in these forests.

A surprising result is that the 15-year-old forest was the only successional stage where the abundance of total carbon displayed a variability greater than 25% and a significant patchy distribution. This is an indication that the 15-year-old forest is the only successional stage where organic matter in the soil is not homogeneously distributed at the within-plot scale. This is unexpected because the 100-year-old was the only successional stage where the total standing litter mass was patchy. Therefore, the patchy distribution of organic matter in early succession does not seem to be associated with pockets of high abundance of present litter resources. These results are not consistent with the findings of Gonzalez & Zak (1994). In a largely undisturbed tropical dry forest in India, organic carbon and forest floor mass were autocorrelated when sampled at a 4 m grain interval, and the location of their patches largely coincided. It may be that the organic matter in the soil in the15-year-old forest in Tarantulas still follows spatial patterns associated with the disturbance process and not with the current vegetation.

The calcium and magnesium kriged maps displayed matching patterns, both in the 15- and the 100-year-old forests, presumably following differences in cation exchange capacity (CEC). However, this variation in CEC was not related to patches of accumulated organic carbon, because the cation patterns did not coincide with patchy distributions of those indicators of organic matter accumulation that were autocorrelated: total carbon in the 15-year-old plot and total litter in the 100-year-old forest. It is possible that the accumulated organic matter in all successional stages is high enough to acidify the soil and to cease to act as a proton trap. Other studies in Tarantulas (Bautista-Cruz *et al.*, 2003) have shown that the soils are acidic (4.1- 4.6) in all successional stages.

Macroinvertebrate community

At the scale studied the macroinvertebrate community appeared to be only consistently patchy in the 100-year-old forest. Few taxa had detectable spatial structures in forests younger than 100 years and the overall community diversity was only patchy in the 100-year-old forest.

Contrary to what was predicted, the variance of macroinvertebrate taxa diversity, both in the litter and soil, diminished with succession. However, the 100-year-old forest was the only successional stage where this variable had a patchy distribution at the 5-24 m scale. This seems at first contradictory, because it was predicted that the scale of patchiness would decrease with the increasing diversity of trees. Therefore, as patches became smaller, variance would increase. The explanation becomes clear when the bubble maps of Shannon's diversity H' are examined (figure 4.9). It is evident in the maps that the index values are more variable in early succession, with some grid intersections with zero diversity and some with values up to 2.46. However, the very small or very large values are not surrounded by intersections with equally small or large values. This indicates that the scale at which diversity spatial structures operate in early succession are smaller than in late succession. Only in the 100-year-old forest spatial structures are large enough to be captured by the scale between 5 and 24 m. In fact, the values in the 100-year-old forest are uniformly large compared to any other stage, but the patches formed by areas of slightly less diversity extend for more than 5 m. Often the CVs of individual taxa abundance followed the same decline with succession (i.e. Chilopoda, Diplopoda and Coleoptera in the litter and Formicidae, Diplurans and Chilopoda in the soil) and most of their distributions were only significantly patchy in late succession. This may be again an indication that the spatial patterning of most taxa abundances in early succession happens at a scale less than 5 m.

It might be thought that the volume of litter present in each monolith would explain part of the difference in macroinvertebrate diversity in the litter community between successional stages. A relationship between the volume of litter and the Shannon Index of macroinvertebrate diversity (as well as its two components taxon richness and evenness) present in a monolith might be expected because there are more feeding resources and a greater amount of habitat with an increase in the volume of litter. However, two pieces of evidence suggest that in this study the volume of litter per monolith is not a determinant of the diversity of macroinvertebrates. First, in Chapter 3 it was shown that the highest mean diversity and number of taxa in the Tarantulas chronosequence were found in the 75- and 100-year-old forests (figure

3.8) while the successional stages with the highest volume of litter per monolith were the 45 and 75-year-old forests (figures 3.4 and 3.5). Second, in this chapter, although the 100-year-old forest was the only successional stage where both the total volume of litter and the diversity of macroinvertebrate taxa had spatially structured distributions at the 5 m scale, the centres of highest and lowest values of taxonomic diversity and highest and lowest volume of litter do not correspond (figures 4.4 and 4.10). While the areas of highest volume of litter in the 100-year-old forest grid were centred on the (1,3), (1,5) and (7,1) intersections, the areas of high taxonomic diversity in the litter were centred on (5,2) and (2,6) intersections.

If taxa abundances and diversity before 100 years of succession are patchy at scales less than 5 m, then there must be different driving forces operating at smaller scales in these forests. It is possible that in successional stages younger than 100 years the diversity of large trees that can influence the spatial distribution of macroinvertebrates at a 5 – 10 m scale is low and the spatial patterns of the understory herbaceous vegetation are the driving force of macroinvertebrate spatial structuring at smaller scales. It has been suggested before that high diversities in the understory and tree vegetation strata promote spatial structures in the soil community at different scales. Ettema & Yeates (2003) found in New Zealand that the nematode community was more strongly aggregated in an old forest than in an adjacent pasture, both at the 0.05 m and the 3 m grain intervals. They suggest that the spatial structuring of the forest nematode community at those two nested scales responds respectively to the understory and tree diversity, which is absent in the pasture.

Also consistent is the suggestion of Saetre & Bååth (2000) that the scale of spatial patterns in the microorganism community composition (PLFA -phospholipid fatty acid- pattern) is larger in forests than in man made environments. In a Norway spruce-birch stand they found that the scale at which the microorganism community forms spatial structures may be an order of magnitude higher than that in agricultural fields (1-11m *vs* < 0.2 m). Further, the spatial structures of the microorganisms in the forest matched the position of trees, particularly spruce trees which had a significant effect on the abundance of 12 PLFA. Some studies do not support the idea that vegetation is the main driver of soil organism spatial structures at the within-plot scale. Görres *et al.* (1998) for example found that the spatial patterns of nematode abundance both in an old growth forest and in an agricultural field in Rhode Island were correlated with the patches of soil moisture content and happened at similar scales (1-10m). While Robertson & Freckman (1995) found in a corn field that nematode trophic groups were aggregated at scales of tens of metres partially associated with edaphic factors.

In this study, an increasing tree diversity through succession was accompanied with an increasing macroinvertebrate community diversity. Simultaneously, the members of the macroinvertebrate community seemed to become spatially structured at a 5-24 m scale in the latest successional stage, particularly in the litter community. One of the mechanisms by which plant diversity can promote spatial structuring in the soil community is through the development of a patchy litter layer. It has been suggested that an important factor that accompanies the heterogeneity of litter resources is the increment in microhabitat diversity, which can promote coexistence through habitat and resource partitioning, releasing some competitive pressure (Anderson, 1978; Amarasekare, 2003). This was demonstrated by Sulkava & Huhta (1998) in a microcosm experiment where the microarthropod species diversity was better maintained when litter had a patchy structure than when it was homogeneous, even if both cases were equivalent in overall chemical quality. Some evidence in the present study points to a patchier distribution of different litter components in the 100-year-old forest compared to earlier successional stages. It is possible that the patchy distribution of the litter resources in the 100-year-old forest is promoting a more diverse and spatially structured litter community.

Another mechanism by which the diversity of the plant community can promote a diverse and spatially structured soil community is through the development of a patchy nutrient environment. As discussed earlier this can happen through production of litter of different qualities and/or through the influence of root activity. The study by Saetre & Bååth (2000) supports this idea. After finding that the spatial structures of the microorganism in the forest matched the position of spruce and birch trees, a comparison with a laboratory experiment showed that the distinct microbial communities under each species were likely to be driven by differences in the quality of organic matter developed in the soil under the canopy of each tree species. In this study the spatial distribution of pine and oak litter seemed to match the distribution of some nutrients in the soil. However, the results do not show a clear trend of increasing patchiness in nutrient abundance with succession, and therefore it seems unlikely that the macroinvertebrate community's patchiness late in succession is tightly bound to the distribution of nutrients in the soil. In fact, while some of the spatial patterns of the litter components and organic matter (total carbon) seem to match those of some macroinvertebrate taxa in different successional stages, none of the nutrient patches appears to coincide with the distribution of any taxon.

Spatial patterns of soil biota have been suggested to be dynamic rather than static over time (Ekschmitt & Griffiths, 1998; Ettema & Wardle, 2002), and often show greater fluctuations than do underlying patterns of abiotic and biotic resources (Görres *et al.*, 1998). In Chapter 6 the temporal variation of soil macroinvertebrate communities in natural populations and within decomposing experimental litter will be considered when studying the single-tree influence on soil biogeochemistry.

The next step

Throughout this chapter I often pointed out that the spatial structures observed the in kriged map of a variable matched the structures in the map of one or two other variables. The statistical test of these multivariate spatial relationships, however desirable, is not a trivial issue. Only very recently formal univariate (or bivariate) tests for spatial patterns in biological and environmental variables have been implemented to incorporate the spatial dimension in the study of ecosystems (Rossi *et al.*, 1992). Even if ecological phenomena are inherently multivariate and spatially structured, because they result from the interaction of multiple processes operating at different scales, the statistical toolkit that allows spatial multivariate relationships to be assessed is currently under development. The recent paper by Wagner (2003) pointed to the necessity of an integrated methodological approach to understand what determines organism community structures in space and proposed the variogram matrix as the framework for the mathematical unification of geostatistics, multivariate data analysis and analysis of variance. This exciting new statistical development will prove immensely useful in understanding the relationship between biodiversity above-ground and below-ground, and will ease the incorporation of the spatial dimension in biodiversity research.

The incorporation of these very recent developments in multivariate spatial statistics into the ecologist statistical toolkit will require some time and therefore it is beyond the scope and timeframe of this thesis to apply them. Instead, I have adopted the approach suggested by Ettema & Wardle (2002) to use spatial analysis to generate hypotheses about the relationship between above-ground and below-ground subsystems, and form the basis upon which mechanisms can be tested through experimentation. Therefore I have broken down this process into two steps. First I have shown in this Chapter that macroinvertebrate taxa and some of the litter components are more structured in space at the within plot scale in late succession. The finding that soil properties and communities are spatially structured at the within-plot scale in the forest where tree diversity is highest leads to the hypothesis that the zone of influence that different tree species create under their canopies generates a mosaic of biogeochemical conditions in the soil. In Chapter 6 I tackle this hypothesis with a field experiment set in the 100-year-old forest. This experiment tests whether the soil biogeochemical activity and litter macroinvertebrate community are determined by the influence that different tree-species exert on the soil beneath their canopy.

Conclusion

This study was limited to one chronosequence and one grid per successional stage, therefore it was not replicated. Because of the intensity involved in grid sampling, it was not possible to replicate the study in such an isolated area. Despite this limitation, results suggest that the increase in tree diversity with succession observed in Mexican TMCF's can be accompanied by an increment in the spatial structuring of litter resources and macroinvertebrate community at the within-plot scale. In contrast, microenvironmental conditions seem to be relatively homogeneous within plots and their heterogeneity does not increase with succession.

The abundance of nutrients was patchy in all successional stages and it did not seem to increase in heterogeneity with succession. The areas of low calcium and magnesium concentration in early succession, and those of phosphorus in late succession seem to match the areas where the litter of pine and oak are respectively most abundant. This suggests that the low concentration of particular nutrients in the litter of different tree species can promote a patchy distribution of those nutrients in the soil.

CHAPTER 5.

A FIELD EXPERIMENT RELATING LEAF DECOMPOSITION AND MACROINVERTEBRATE COMMUNITIES ALONG A SUCCESSIONAL GRADIENT

Introduction

The rate of litter decomposition in forests is regulated by climatic factors, the physical properties of the soil, the chemical quality of resources and by interactions between organisms, which operate in a hierarchical manner (Swift *et al.*, 1979; Lavelle *et al.*, 1993). The influence of factors low in this hierarchy depends on those factors higher in the hierarchy to be non-limiting for decomposition. For example, in tropical regions with relatively aseasonal climates, litter quality and interactions between decomposer organisms play a prime regulatory role in decomposition rate (Lavelle *et al.*, 1993). Although high altitude TMCFs are located within the tropical region, climatic factors are thought to exert an important control over biological processes (Vitousek *et al.*, 1994; Bruijnzeel & Proctor, 1995; Waide *et al.*, 1998). Limited solar radiation caused by high cloud cover may have a cascading effect resulting in low productivity, nutrient poor soils, low litter quality and low rates of nutrient cycling and decomposition (Bruijnzeel & Veneklaas, 1998).

In Chapter 3 it was shown that logging disturbance in the Oaxacan TMCF significantly alters the geochemical characteristics of mature forests. With the opening of the canopy, soil temperature increases, nutrients become more available in the soil and macroinvertebrate community composition is altered. After disturbance, conditions start to slowly recover. Soil temperature decreases, soil organic matter accumulates, nutrients availability declines and the number of soil macroinvertebrate taxa increases. In this chapter I first explore whether these successional changes that occur below-ground are associated with a decline of decomposition rate and reduced nutrient release from litter. Then, I analyse whether changes in the macroinvertebrate community observed through succession are a result of changes in the quality of decomposing litter or are driven by other soil or environmental characteristics inherent to each successional stage.

The predicted lower decomposition rate in late succession would contradict the diversity-function relationship hypothesis (Rivet hypothesis; Ehrlich & Ehrlich, 1981) because an increased diversity of tree genera and litter resources as well as a richer macroinvertebrate community would be associated with lower rates of ecosystem process (e.g. decomposition rates and release of nutrients). Given that macroinvertebrates exert an indirect effect on decomposition (Swift *et al.*, 1979; Seastedt, 1984) and the magnitude of this effect depends on the limitation of climatic factors (higher in the control hierarchy), it is plausible that, independently of the rate of the process, decomposing litter from late successional tree species is associated with a richer macroinvertebrate community than the litter from pioneer trees. In this sense late successional trees may serve as a *keystone* (Vitousek & Hooper, 1993) group as far as sustaining soil fauna diversity in TMCFs.

Decomposing litter from late successional trees may sustain a richer macroinvertebrate community because (1) the litter resources that the diverse canopy provides promote an equally diverse decomposer food-web or (2) there is some character associated with the stress-tolerant life history of late successional tropical trees in Mexican TMCFs (González-Espinosa *et al.*, 1991; Quintana-Ascencio & González-Espinosa, 1993; Galindo-Jaimes *et al.*, 2002) that favours the development of a rich macroinvertebrate community. Possibly the low quality litter provided by stress-tolerant tree species (Grime & Anderson, 1986) accumulates and semi-decomposed organic matter builds-up down the soil profile. In the long term a richer decomposer food-web may develop because a variety of resource qualities develop as a result of the prolonged stages of decomposition.

In terms of decomposition, both the mixing of litter of diverse quality and life-history traits of plant species have been shown to have an effect on the decomposition rate and nutrient mineralisation in litter. Litter mixtures have strong positive or negative effects on decomposition rate depending on the plant species involved (Wardle *et al.*, 1997b; Wardle *et al.*, 2003). High nutrient content in the litter of one species can enhance decomposition rate in the litter of an other that is nutrient poor, but inhibitors in poor quality resources can inhibit decomposition in neighbouring resources (Seastedt, 1984). On the other hand, as a result of the high cost of leaf production in nutrient-limited environments, stress-tolerant plants produce leaves that are well protected with herbivore deterrents. These carbon-rich complex molecules are still present after leaf shedding and reduce decomposition rate (Grime & Anderson, 1986; Cornelissen, 1996; Grime *et al.*, 1996)

A third explanation for the increase in soil macroinvertebrate richness during succession may be a long-term effect of forest disturbance. This can happen through two mechanisms. First it may be that the fauna was severely affected by the previous logging event and complete re-colonisation takes more than 100 years of succession, or second, soils are known to retain the history of the vegetation for long periods of time and organisms may still be responding to the effects of the original perturbation (Anderson, 2000). Therefore, the composition of the soil macroinvertebrate community may be determined by soil characteristics that are a result of historical events rather than of current environmental and nutrient conditions.

This chapter presents the results of a decomposition experiment in the Tarantulas chronosequence that was designed to explore the relationship between successional trends in the macroinvertebrate community and litter decomposition. The leaves of an early-successional conifer (*Pinus chiapensis*) and a late-successional broad leaved tree species (*Persea americana*) were set to decompose in each of the successional stages with the following objectives:

1. To determine if the return of the TMCF to a more nutrient-limited condition through secondary succession is associated with the predicted slow-down of decomposition and nutrient release from litter, independently of litter quality.

2. To determine if a richer macroinvertebrate community colonises the leaves of a late successional species than an early successional species, independently of the successional stage where they are placed.

3. To determine whether the observed similarity of the macroinvertebrate community in intermediate stages of succession (15- to 45-year-olds) is associated with the dominance of pine litter in those stages.

Methods

The experiment presented in this chapter was carried out in the middle of sixteen randomly chosen squares formed by the grid in each successional stage of the Tarantulas chronosequence (figure 2.5). In each of these experimental points, two decomposition boxes were buried under the litter layer on 21-22 May 2001. One of the boxes contained pine (*Pinus chiapensis*) needles and the other avocado (*Persea americana*) leaves, both collected fresh from trees and oven-dried to constant mass. The litter boxes in four experimental points were retrieved 28, 56, 112 and 210 days later (19-29/Jun/01, 17-18/Jul/01, 17-18/Oct/01 and 16-17/Jan/02).

Details of decomposition box construction, extraction and processing, macroinvertebrate sorting and identification and chemical analysis of experimental litter can be found in Chapter 2.

Statistical Analysis

Details of the statistical analysis of decomposition rate, chemistry evolution and macroinvertebrate diversity in decomposition boxes can be found in Chapter 2.

Relationship between macroinvertebrate communities and experimental treatments

CCAs and Variance Partitioning were performed in three steps:

(1) A general ordination (followed by Variance Partitioning) of the macroinvertebrate communities (found in retrieved decomposition boxes) with respect to experimental treatments and leaf chemistry. Variables included successional stage (four dummy variables), collection date (four dummy variables), experimental leaf species (two dummy variables) and leaf nutrient,

RF and ASF contents (eight variables). Because of the complexity of this analysis no biplots were presented.

(2) Separate ordinations per successional stage of the box macroinvertebrate community with respect to collection date, leaf species and leaf nutrient, RF and ASF contents. Biplots were drawn for each of these ordinations.

(3) Separate ordinations per collection date of the box macroinvertebrate community with respect to successional stage, leaf species and leaf nutrient, RF and ASF contents. Biplots were drawn for each of these ordinations.

Details on the CCA and Variance Partitioning procedures can be found in Chapter 2. In this chapter the Monte-Carlo randomisation tests are presented along with all CCAs. These randomisations were performed considering each of the experimental boxes as independent observations. However, placing the boxes in pairs (*P. americana* and *P. chiapensis* boxes) within grids introduces a block effect that is not considered in the randomisation test. Despite this limitation, the Monte-Carlo tests were useful as a preliminary means of testing the robustness of the patterns shown by the CCAs for spurious effects. Therefore randomisation tests should be interpreted conservatively bearing in mind that, for the effect of successional stage, individual boxes in each pair are pseudo-replicates and only 16 real replicates exist per successional stage.

Results

Mass loss and chemical evolution during decomposition of Persea americana leaves and Pinus chiapensis needles in different successional stages

Mass loss

A three-way analysis of variance showed that there was a significant interaction between the effects of collection date and species of leaf on the mass remaining in the decomposition boxes (F=4.01, d. f.=3, P<0.01)[1]. This interaction is explained by the absence of differences between leaf species at the first two collection dates (after

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[1] ANOVA tables including the error degrees of freedom for all F ratios, are given in Appendix CH5.

28 and 56 days). However, at the third and fourth collection dates (after 112 and 220 days) the boxes containing *Pinus chiapensis* needles had less mass remaining than those containing *Persea americana* (figure 5.1).

The difference in decomposition rate between *Pinus chiapensis* and *Persea americana* is reflected in the difference in exponential decay constants (*k*), 0.97 for *P. americana* and 1.14 for *P.chiapensis*. However, the asymptote for both species curves appears to be well beyond zero (figure 5.1), denoting that the litter is formed by two components, one that decays exponentially in the first year of decomposition and a second that is resistant to decay in this initial phase.

The successional stage where decomposition boxes were placed did not have a significant effect on the mass remaining in decomposition boxes for any collection date or species of leaf (figure 5.2).

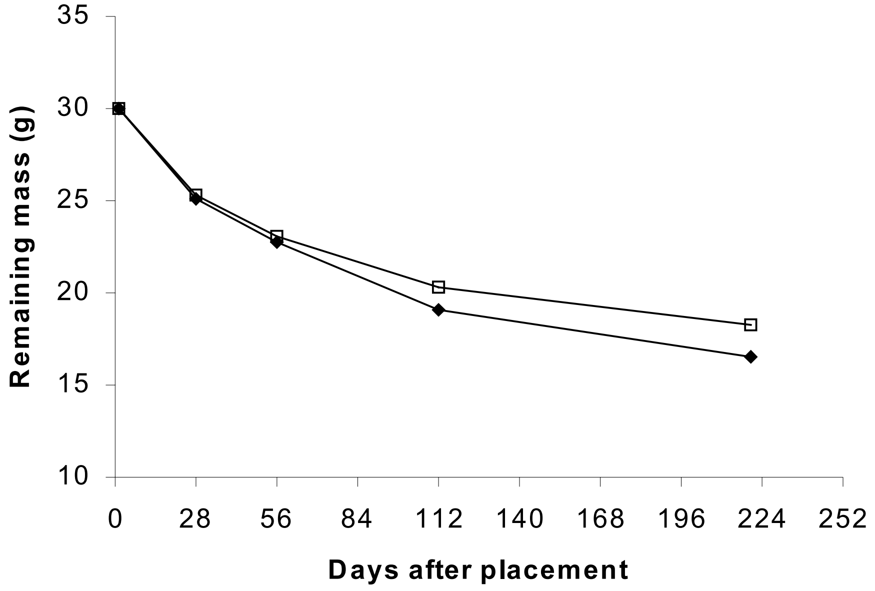


Figure 5.1 Mass loss through time in experimental decomposition boxes. Values for different successional stages are pooled, Mean values and regression lines are coded by the following symbols: *Persea americana* (opened symbols) and *Pinus chiapensis* (closed symbols). Exponential decay constants (*k*) are 0.97 for *P. americana* boxes and 1.14 for *P.chiapensis* boxes.

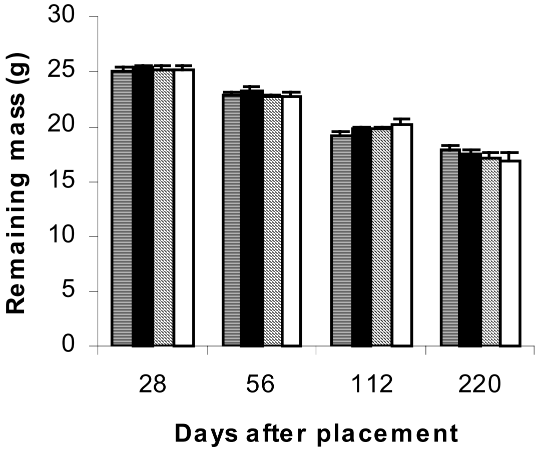


Figure 5.2 Mass loss through time in experimental decomposition boxes. Values for different leaf species are pooled and boxes retrieved from different successional stages are coded by the following bar filling: 15-year-old forest (dark grey), 45-year-old forest (black), 75-year-old forest (light gray) and 100-year-old forest (clear). Values presented are means + standard error. Sample size (n) is 8 per collection date in each successional stage, except for the collection made after 210 days where n= 7 for the 100-year-old forest and n=6 for the 75-year-old forest.

Chemical evolution

Table 5.1 presents a summary of the evolution of nutrient concentration in experimental leaves during the decomposition experiment. For the sake of clarity only mean values of nutrient concentration and percent of initial content are presented. The standard deviations associated with the nutrient concentrations are presented in a separate table in Appendix CH5 (table CH5.11). This dispersion around the treatment means is probably a result of three factors: (1) unaccounted differences between the local environments where the boxes were placed, (2) changes in the chemistry of the litter during the handling period and (3) errors associated with the measurements performed during the laboratory analyses.

The percentage of initial nutrient content that remained at the time of collection was estimated by dividing the final nutrient mass in each sample by the mean nutrient content in four samples of undecomposed leaves. Therefore the error associated with the measurement of initial nutrient content was not considered in this estimate. This assumption was considered to be reasonable since the variation around the mean initial concentrations was moderate for most nutrients (see figure 5.3). The percentages of initial concentration presented in table 5.1 and described below are intended to explore in what stages of the experiment mineralisation and immobilisation most probably occurred. Values in table 5.1 should not be considered as precise comparisons, but rough estimates of initial vs. final nutrient content. The statistical tests of the effect of experimental treatments on the chemical quality of decomposing leaves are discussed later in this section.

Boxes with different foliar species differed in terms of the absolute nutrient content through the experiment (table 5.1, figure 5.3). In boxes containing *P. americana* no net immobilisation (increments beyond 100% or initial absolute content) of nitrogen or phosphorus occurred, and by the end of the experiment substantial amounts of both elements had been released through leaching (losses up to 20% of initial amount) and mineralisation (losses beyond 20% of initial amount). In contrast, in boxes containing *P. chiapensis* nitrogen was immobilised in all successional stages (except the 15-year-old forest) at some point during the experiment. In contrast phosphorus was increasingly immobilised throughout the experiment. Apart from subtle differences, cations were lost from both foliar species at a steady pace as the experiment proceeded. In the boxes containing *P. americana* retrieved in the first collection date, the content of sodium increased beyond the initial net amount in the 75- and 100-year old forests (table 5.1). In both foliar species RF and ASF net content increased rapidly beyond 100% of the initial amount and it only started to be lost again by the last collection date. The net gains in ASF and RF were more pronounced in *P. americana* (table 5.1).

As has already been discussed for abscised leaves in Chapter 3, in this chapter the proportions found for acid soluble (ASF) and remaining (RF) fibre fractions in the fresh leaves of *P. chiapensis* and *P. americana* do not appear to correspond to the conventional fractions found by other authors using the same extraction technique (Van Soest, 1994). For example Berg and Tamm (1991) found that fresh needles of

Picea abies contained 20.8% of RF (lignin) while in this study the fresh needles of *P. chiapensis* had a mean 48.3% of RF. Similarly, Kwabbiah *et al.* (2003) found that the fresh leaves of six species of broad leaves contained between 8% and 22% of RF (lignin) while *P. americana* in this study contained a mean RF of 43%. This discrepancy in the results was discussed in detail in Chapter 3 (p.102 and table CH3.19 in Appendix CH3) and therefore will not be expanded further here. It should be sufficient to say that it is evident that the range of ASF values in this study is considerably lower and the one of RF considerably higher than the concentrations reported in the literature. Therefore ASF and RF have only been interpreted as representing fibrous fractions of the litter that are relatively more and less labile.

Seventy percent of the variance in the nutrient, ASF and RF concentration variables can be represented by three principal components (from here on named foliar quality components; table 5.2). The first foliar quality component (PCA1) extracted 36.2% of the total variance and was mostly determined by the contrasting variations in carbon in one direction and potassium, calcium and sodium in the other (see factor loadings in table 5.2). The second component (PCA2) extracted 22.1% of the total variance and represented mainly the variation in nitrogen and RF in one direction and magnesium and sodium in the other. The third component (PCA3) accounted for 11.6 % of the variance and was determined mainly by the variation in phosphorus in one direction and to a lesser extent the variation in RF in the other.

All experimental treatments had significant effects on the three foliar quality components and in many cases effects were interactive. The mean value of PCA1 was higher for decomposition boxes containing *Pinus chiapensis* than for the ones containing *Persea americana* (F=954.3, d.f.=1, P<0.0001; figure 5.4a). This implies that initial differences in chemical composition of the leaves (figure 5.3 and table 5.1) were sustained through out the experiment, *P. americana* leaves always had higher concentrations of potassium, calcium and sodium and lower concentration of total carbon than *P. chiapensis* needles. The placement of experimental boxes in different successional stages had no significant effect on the value of PCA1 in boxes containing *P. chiapensis*, but had a significant effect on the ones containing *P.*

Persea americana																			
	Days	**C**		**N**		**P**		**Na**		**K**		**Ca**		**Mg**		**ASF**		**RF**	
Initial		g.	%	g	%	cmol	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	g	%	g	%
	0	15.86	53.33	0.46	1.56	0.07	2.61	0.02	0.55	0.74	24.8	2.72	91.15	1.00	33.78	0.75	2.51	12.78	42.97
Forest age		%of I.	%	%of I.	%	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	%	%of I.	%
15-year-old	28	85.62	53.72	92.63	1.70	117.55	3.61	72.34	0.47	67.78	19.78	54.00	57.91	79.94	31.77	133.03	3.93	122.73	62.04
	56	77.28	53.32	80.38	1.62	50.93	1.72	60.60	0.43	57.88	18.57	39.85	46.99	88.76	38.79	116.31	3.78	113.44	63.07
	112	70.09	54.60	79.69	1.82	66.44	2.53	46.62	0.37	18.73	6.79	29.09	38.73	70.66	34.87	128.49	4.71	123.22	77.35
	220	62.09	53.62	63.47	1.60	79.30	3.35	49.03	0.44	7.77	3.12	27.25	40.22	59.18	32.37	121.18	4.93	102.55	71.36
45-year-old	28	87.6	54.03	82.96	1.50	65.98	1.99	64.85	0.41	66.39	19.04	52.66	55.51	84.12	32.86	131.06	3.80	122.85	61.05
	56	79.86	53.52	87.41	1.71	78.21	2.56	85.07	0.59	70.41	21.94	54.86	62.84	91.35	38.77	103.46	3.26	117.16	63.26
	112	67.23	53.90	85.78	2.01	68.08	2.67	42.06	0.35	22.92	8.54	37.15	50.90	70.12	35.61	128.16	4.84	129.25	83.49
	220	62.63	53.50	70.24	1.76	100.72	4.21	33.35	0.29	11.32	4.50	31.96	46.66	72.84	39.41	97.74	3.93	98.35	67.69
75-year-old	28	83.04	52.93	87.73	1.64	70.49	2.20	108.06	0.71	67.94	20.14	70.95	77.29	84.60	34.16	154.8	4.64	106.4	54.64
	56	75.2	52.17	79.07	1.60	59.72	2.03	46.73	0.33	53.43	17.24	52.65	62.43	95.80	42.10	106.93	3.49	106.66	59.62
	112	68.84	54.10	93.59	2.15	65.45	2.52	35.84	0.29	10.26	3.75	37.09	49.82	61.85	30.79	142.55	5.27	130.17	82.43
	220	60.7	53.62	67.94	1.76	98.83	4.27	46.24	0.42	11.23	4.61	31.29	47.24	61.75	34.55	93.24	3.88	94.9	67.55
100-year-old	28	85.84	53.70	85.4	1.56	70.85	2.17	94.65	0.61	60.32	17.55	54.83	58.63	93.07	36.88	99.29	2.92	124.07	62.54
	56	75.77	52.95	98.48	2.01	51.92	1.78	40.17	0.29	39.33	12.78	38.86	46.42	69.34	30.69	140.16	4.61	110.95	62.47
	112	71.5	54.34	78.08	1.74	68.43	2.54	28.66	0.22	6.49	2.29	34.18	44.40	62.84	30.25	199.2	7.12	112.88	69.12
	220	63.7	55.43	62.78	1.60	104.10	4.43	31.44	0.28	5.98	2.42	33.90	50.41	54.74	30.17	99.64	4.08	97.33	68.23

Table 5.1 Mean concentration and remaining absolute amount of nutrients and sodium, RF and ASF in experimental leaves at the time of recovery. Treatments consisted of forest age (15, 45, 75 and 100 years old), collection time (28, 56, 112 and 210 days after placement) and species of leaf contained in the box (*Persea americana* and *Pinus chiapensis*). The remaining amount of nutrients is expressed in terms of percentage of the initial absolute quantity in original 30 g litter sample (%of I.), except for the initial value, where it is expressed in grams or centimoles. Continues in the next page.

Pinus chiapensis

	Days	C		N		P		Na		K		Ca		Mg		ASF		RF	
Initial		g.	%	g	%	cmol	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	cmol	cmol.Kg^{-1}	g	%	g	%
	0	15.61	56.24	0.35	1.26	0.05	1.97	0.01	0.31	0.30	10.84	0.72	25.93	0.22	7.86	0.54	1.93	13.41	48.32
Forest age		%of I.	%	%of I.	%	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	cmol.Kg^{-1}	%of I.	%	%of I.	%
15-year-old	28	88.75	55.97	92.15	1.30	78.35	1.73	167.37	0.58	88.53	10.76	55.53	16.15	92.64	8.17	119.54	2.59	124.14	67.26
	56	81.09	55.51	91.51	1.40	74.24	1.78	143.27	0.54	76.88	10.14	50.90	16.06	96.92	9.27	131.11	3.08	114.30	67.22
	112	65.53	56.33	79.19	1.53	122.32	3.68	102.77	0.49	35.12	5.82	49.88	19.77	86.49	10.39	138.12	4.07	104.53	77.20
	220	62.46	55.96	76.62	1.54	106.45	3.34	102.41	0.51	52.12	9.00	30.55	12.62	62.98	7.89	145.86	4.48	91.62	70.53
45-year-old	28	89.81	56.22	99.37	1.39	78.25	1.72	123.81	0.43	89.20	10.76	57.50	16.60	88.44	7.74	135.75	2.92	126.73	68.16
	56	82.14	56.12	93.70	1.43	79.99	1.91	128.46	0.48	70.17	9.24	62.62	19.73	85.04	8.12	151.84	3.56	115.92	68.05
	112	70.65	56.02	108.38	1.93	105.45	2.93	80.63	0.35	34.76	5.31	35.39	12.94	84.59	9.37	121.89	3.32	116.99	79.70
	220	58.90	56.37	71.57	1.53	136.49	4.58	76.96	0.41	15.21	2.81	32.52	14.35	68.78	9.20	88.66	2.91	76.14	62.60
75-year-old	28	91.83	56.24	91.67	1.26	85.73	1.84	109.92	0.37	92.87	10.96	56.26	15.89	82.99	7.10	131.05	2.75	125.63	66.11
	56	81.77	56.25	95.46	1.47	70.46	1.70	131.41	0.50	64.03	8.49	45.16	14.32	84.79	8.15	122.55	2.89	114.80	67.85
	112	69.64	56.47	110.72	2.01	103.61	2.94	81.32	0.36	30.57	4.78	52.99	19.81	84.37	9.56	98.52	2.74	100.57	70.07
	220	58.99	56.56	69.34	1.49	125.16	4.20	90.07	0.48	13.18	2.44	27.72	12.26	71.50	9.58	115.90	3.81	79.69	65.64
100-year-old	28	89.92	55.98	98.00	1.37	100.71	2.20	139.68	0.48	92.00	11.04	61.73	17.72	96.52	8.40	152.96	3.27	120.58	64.49
	56	81.96	56.03	104.94	1.61	71.11	1.70	87.17	0.33	75.80	9.99	47.83	15.07	91.88	8.78	169.16	3.97	109.78	64.48
	112	69.42	56.08	75.91	1.37	77.03	2.18	60.43	0.27	23.23	3.62	35.76	13.32	74.94	8.46	151.09	4.19	95.48	66.26
	220	57.19	56.25	76.43	1.68	132.89	4.58	83.29	0.45	24.38	4.62	28.39	12.88	67.55	9.29	128.43	4.34	75.62	63.91

Table 5.1 Continuation

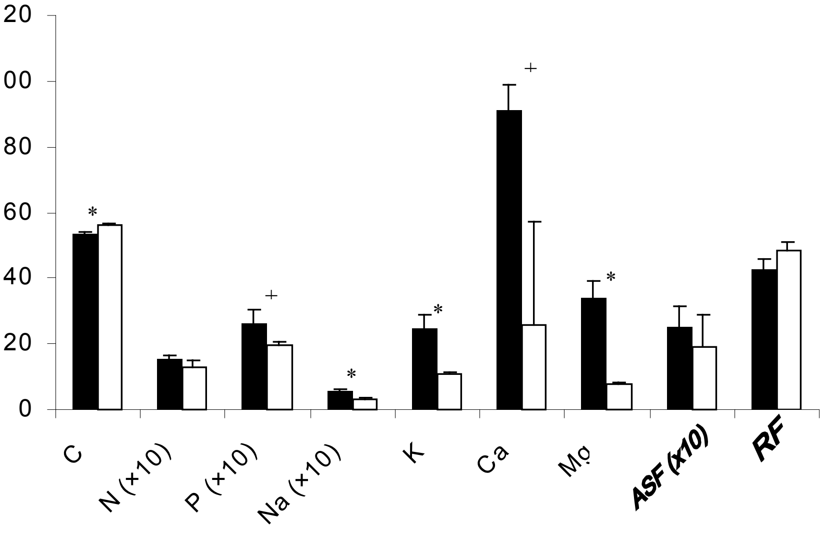


Figure 5.3 Graph illustrating initial differences in chemical composition of experimental leaves. Variables included were Carbon (%), Nitrogen (%), P (cmol.kg^{-1}), Mg^{++}(cmol.kg^{-1}), Na $^{+}$(cmol.kg^{-1}), K^{+}(cmol.kg^{-1}), Ca^{++}(cmol.kg^{-1}), ASF (%) and RF (%). * indicates significant differences between foliar species at the level of $P<0.05$ with Bonfferroni's adjustment for multiple comparisons. + Indicates those differences that were significant without considering multiple testing but became non-significant after Bonfferoni's adjustment. Values presented are means ± standard error. (×10) indicates that, for presentation reasons, the chemical concentration has been multiplied by 10. Dark bars represent *Persea americana* leaves and light bars *Pinus chiapensis* needles.

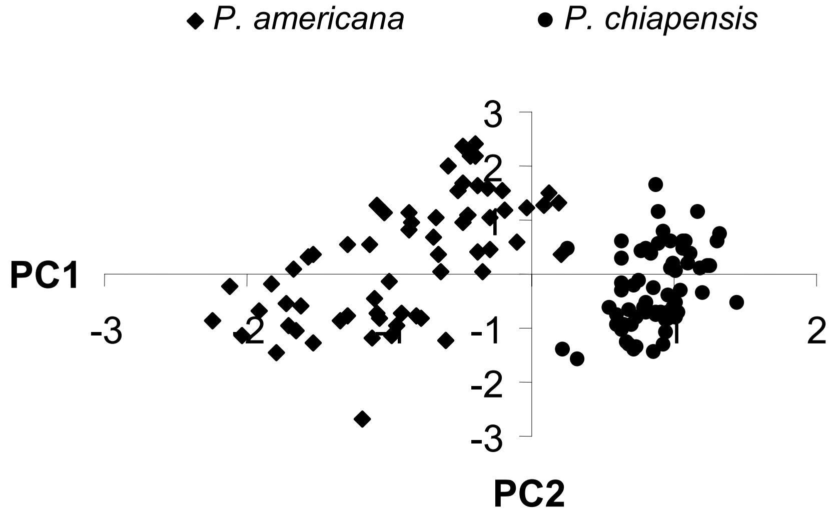
	Factor loading		
Variable	**Axis 1**	**Axis 2**	**Axis 3**
Total carbon	**0.938**	-0.073	-0.005
Total nitrogen	-0.328	**0.662**	**0.278**
Total phosphorus	0.092	0.413	**-0.789**
Mg^{++}	-0.108	**-0.627**	-0.102
Na+	**-0.622**	**-0.595**	0.157
K^{+}	**-0.948**	0.049	-0.053
Ca^{++}	**-0.903**	0.244	0.012
ASF	-0.232	0.474	**-0.251**
RF	0.317	**0.585**	**0.496**
Eigenvalues	3.261	1.988	1.0463
% of variance extracted	36.2	22.1	11.6

Table 5.2. Principal Component Analysis of the nutrients, RF and ASF concentrations in the leaves remaining in experimental boxes at the time of recovery. Boxes from all treatments included. Treatments consisted of forest age (15, 45, 75 and 100 years old), collection time (28, 56, 112 and 210 days after placement) and species of leaf contained in the box (*Persea americana* and *Pinus chiapensis*). A correlation matrix was used. The four highest absolute values of factor loadings highlighted in bold numbers.

americana leaves (F=5.50, d.f.=3, P<0.01; figure 5.5a). PCA1 increased with succession up to the 75-year-old forest and then decreased again in the 100-year-old forest. In consequence the concentrations of potassium, calcium and sodium tended to be higher and total carbon lower in *P. americana* boxes placed in mid-successional stages (table 5.1). The effect of collection date on PCA1 also differed between boxes containing different foliar species (F=13.40, d.f.=3, P<0.0001; figure 5.6a). There were no differences between collection dates on the value of PCA1 in boxes containing *P. chiapensis* needles. In contrast, the boxes containing *P. americana* that were recovered on the first two collection dates had even lower values of PCA1 than those recovered in the second two collection dates (figure 5.6a). In consequence the concentration of potassium, calcium and sodium decreased in the *P. americana* boxes as the experiment proceeded. Simultaneously, the concentration of total carbon in these boxes increased (table 5.1)

The value of PCA2 was significantly higher in boxes extracted in the second half of the experiment than in those extracted in the first half (F=37.05, d.f.=3, P<0.0001). Therefore, the concentration of nitrogen and RF increased as the experiment proceeded and the concentrations of magnesium and sodium decreased (table 5.1, figures 5.4b, 5.5b and 5.6b;). There was no overall significant effect of successional stage on PCA2. However there was particularly high value of PCA2 in the boxes recovered from the 100 year old forest after 56 days compared with the boxes recovered at the same date from other successional stages (figure 5.5b). The effect of collection date on PCA2 interacted with the effect of foliar species (F=5.46, d.f.=3, P<0.01). At the first two collection dates there was no difference in the value of PCA2 between boxes containing different foliar species. In the second half of the experiment the value of PCA2 was higher for *P. americana* (figure 5.6b). In consequence, the concentration of nitrogen and RF increased more in *P. americana* leaves than in *P. chiapensis* needles as the experiment proceeded. Simultaneously, the concentrations of magnesium and sodium decreased significantly more in *P. americana* than they did in *P. chiapensis* (table 5.1).

(a)



(b)

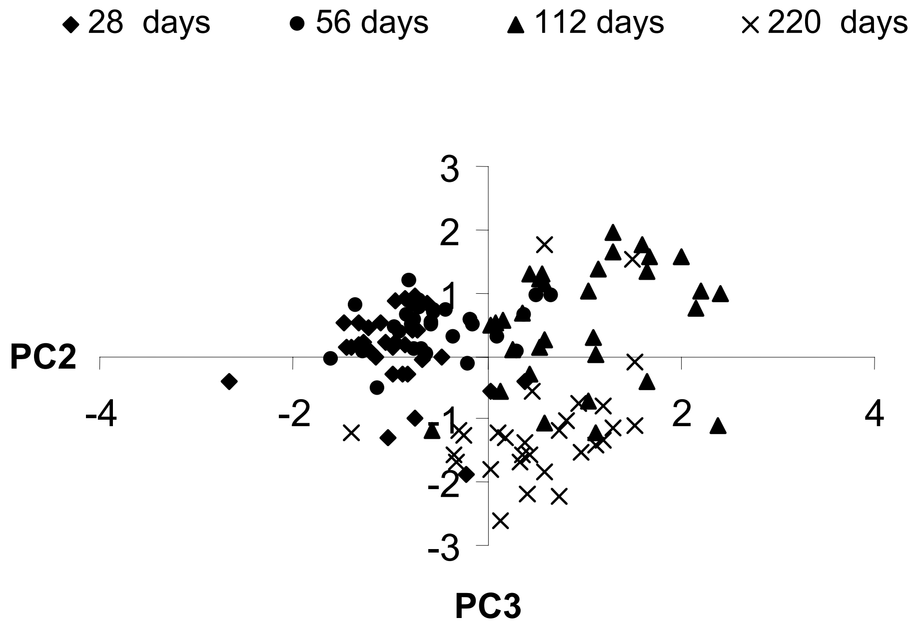


Figure 5.4 Ordination diagrams based on principal component analyses of nutrients, ASF and RF concentrations in decomposition boxes at the time of collection. Variables included in the components were Carbon (%), Nitrogen (%), P (cmol.kg^{-1}), Mg^{++}(cmol.kg^{-1}), Na $^{+}$(cmol.kg^{-1}), K^{+}(cmol.kg^{-1}) and Ca^{++}(cmol.kg^{-1}). Sample scores plotted for (a) principal component one (PCA1) against PCA2 and (b) PCA2 against PCA3. Leaf species (a) and collection date (b) are symbol coded.

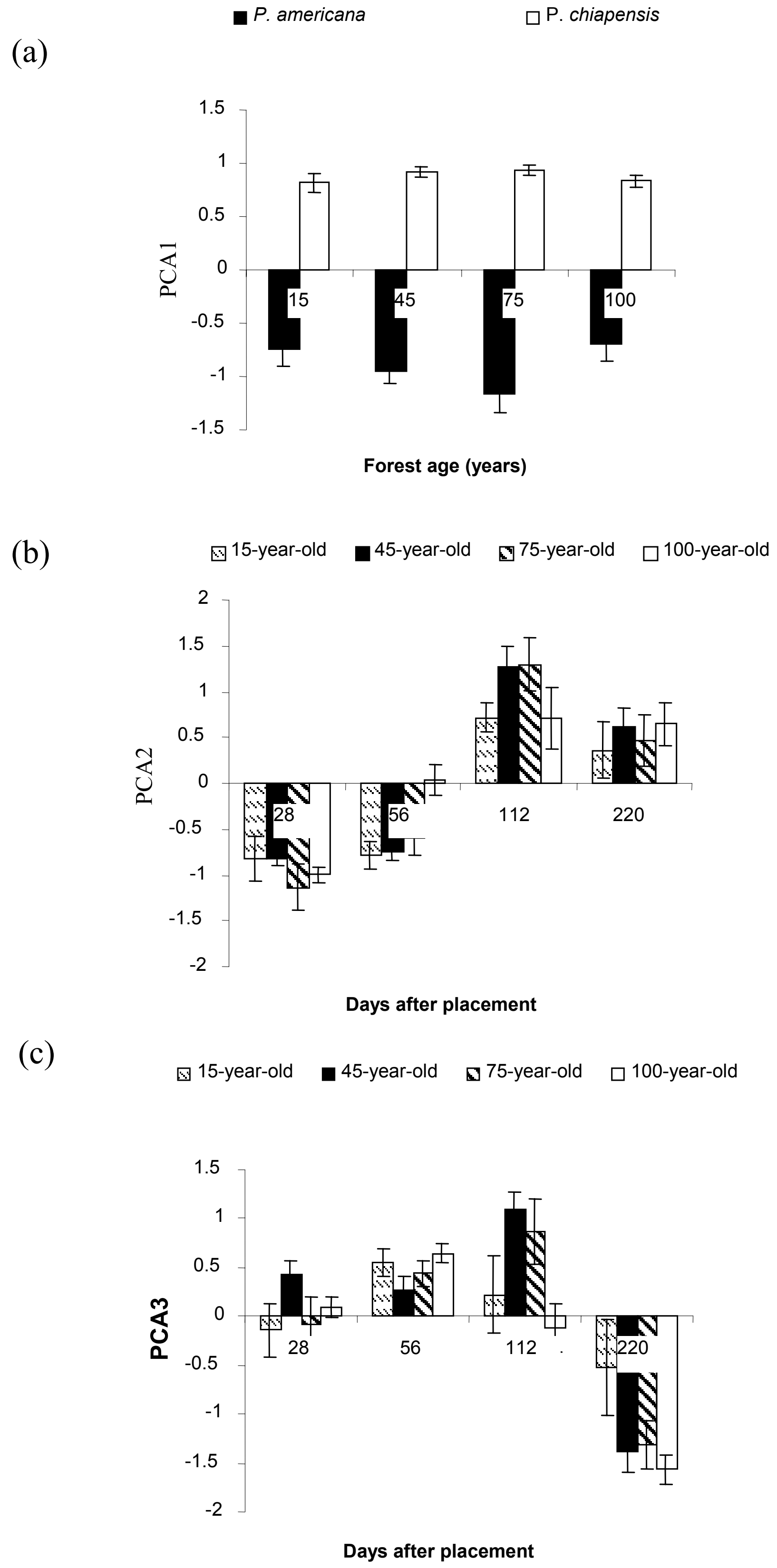


Figure 5.5 Graphs illustrating significant interactive effects of successional stage with other experimental treatments on the first three principal components formed by nutrients, ASF and RF concentration variables in decomposition boxes. Variables included in the PCA were Carbon (%), Nitrogen (%), P (cmol.kg^{-1}), Mg^{++}(cmol.kg^{-1}), Na $^{+}$(cmol.kg^{-1}), K^{+}(cmol.kg^{-1}) and Ca^{++}(cmol.kg^{-1}). Significant interaction effects of successional stage with (a) foliar species (on PCA1) and (b-c) collection date (on PCA2 and PCA3) are presented. Values presented are means ± standard error. Sample size (n) in graph (a) is 16 per foliar species in each successional stage except for the 75-year-old (*P. americana* and *P. chiapensis*) and the 100-year-old (*P. americana* only) forests where n=15. In graphs (b) and (c) n=8 except for the collection after 210 days in the 75-year-old and the 100-year-old forests where n= 6 and 7 respectively.

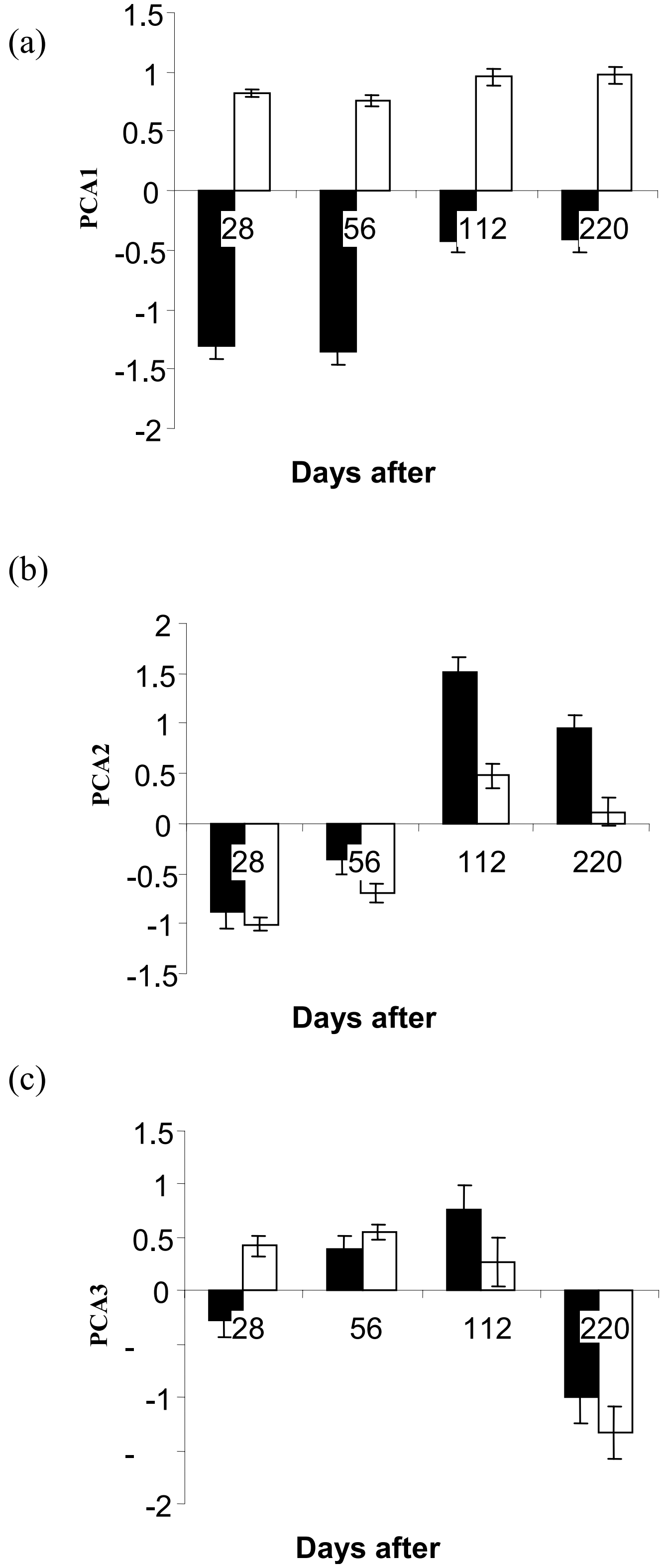


Figure 5.6 Graphs illustrating significant interactive effect between collection date and leaf species on the first three (a-c) principal components formed by nutrients, ASF and RF concentration variables in decomposition boxes. Variables included in the PCA were Carbon (%), Nitrogen (%), P (cmol.kg^{-1}), Mg^{++}(cmol.kg^{-1}), Na $^{+}$(cmol.kg^{-1}), K^{+}(cmol.kg^{-1}) and Ca^{++}(cmol.kg^{-1}). Dark bars represent decomposition boxes containing *Persea americana* leaves and light bars boxes with *Pinus chiapensis*. Values presented are means ± standard error. Sample size (n) is 16 per foliar species in collection date except for the collection after 220 days where n=14.

Experimental boxes extracted at the last collection date displayed lower values of PCA3 than at the other collection dates (F=12.92, d.f.=3, P=0.001; figure 5.4c, 5.5c and 5.6c). This was driven by the concentration of phosphorus increasing and that of RF lowering between 112 and 220 days collections. There was no overall significant effect of successional stage on PCA3. However, there appears to be a higher value of PCA3 in the experimental boxes recovered from the 45-year-old forest after 112 days than the ones recovered in the 100-year-old forest on the same date (figure 5.5c). This seems to be mainly a response to the higher concentration of RF in the boxes retrieved from the 45-year-old forest and the lower one in the ones retrieved from the 100-year-old (table 5.1). There was a significant interactive effect between collection date and foliar species on PCA3 (F=4.91, d.f.=3, P<0.01), probably owing to the lower concentrations of P and RF recorded in boxes containing *P. americana* collected on the first date compared to those containing *P. chiapensis* collected on the same date (table 5.1; figure 5.6c).

Macroinvertebrate communities in experimental decomposition boxes

Number of macroinvertebrate taxa, equitability and Shannon's Diversity Index

The number of macroinvertebrate taxa present in experimental decomposition boxes was consistently higher in those boxes that contained *Persea americana* leaves (F=18.59, d.f.=1, P<0.0001) and increased with the number of days that the box spent in the field (F=7.48, d.f.=3, P<0.01 and figures 5.7a and 5.8a). However, the number of taxa was not significantly different between successional stages and there were no interactions between the effects of successional stage, collection date and leaf species.

The equitability and Shannon's diversity indices of macroinvertebrate taxa present in decomposition boxes were always higher for boxes containing *Persea americana* (F=9.00, d.f.=1, P<0.01 for equitability and F=9.55, d.f.=1, P<0.01 for Shannon's diversity index; figure 5.7 b and c) and there was no overall significant effect of successional stage or collection date on these variables.

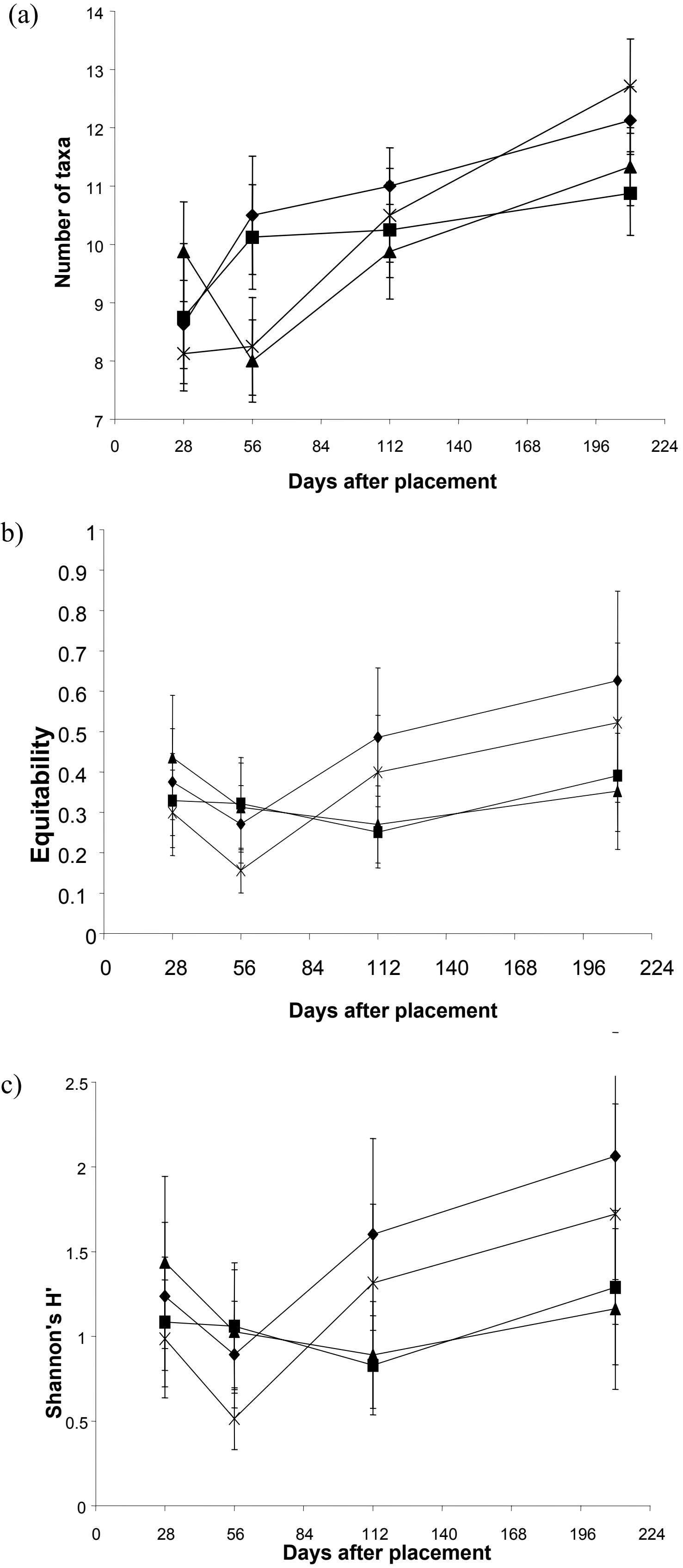


Figure 5.7. (a) Number, (b) equitablility and (c) Shannon's H' of macroinvertebrate taxa present in decomposition boxes that had been buried in forest soils for different lengths of time (28, 56, 112 and 210 days). Values for different successional stages are coded by the following symbols: × 100-year-old forest, ▲75-year-old forest, ■ 45-year-old forest and ♦ 15 year-old-forest. Boxes containing *Persea amiericana* leave*s* and *Pinus chiapensis* needles are pooled. Values presented are mean ± standard error. Sample size (n) is 8 per collection date in each successional stage, except for the collection after 210 days in the 75-year-old and the 100-year-old forests where n= 6 and 7 respectively.

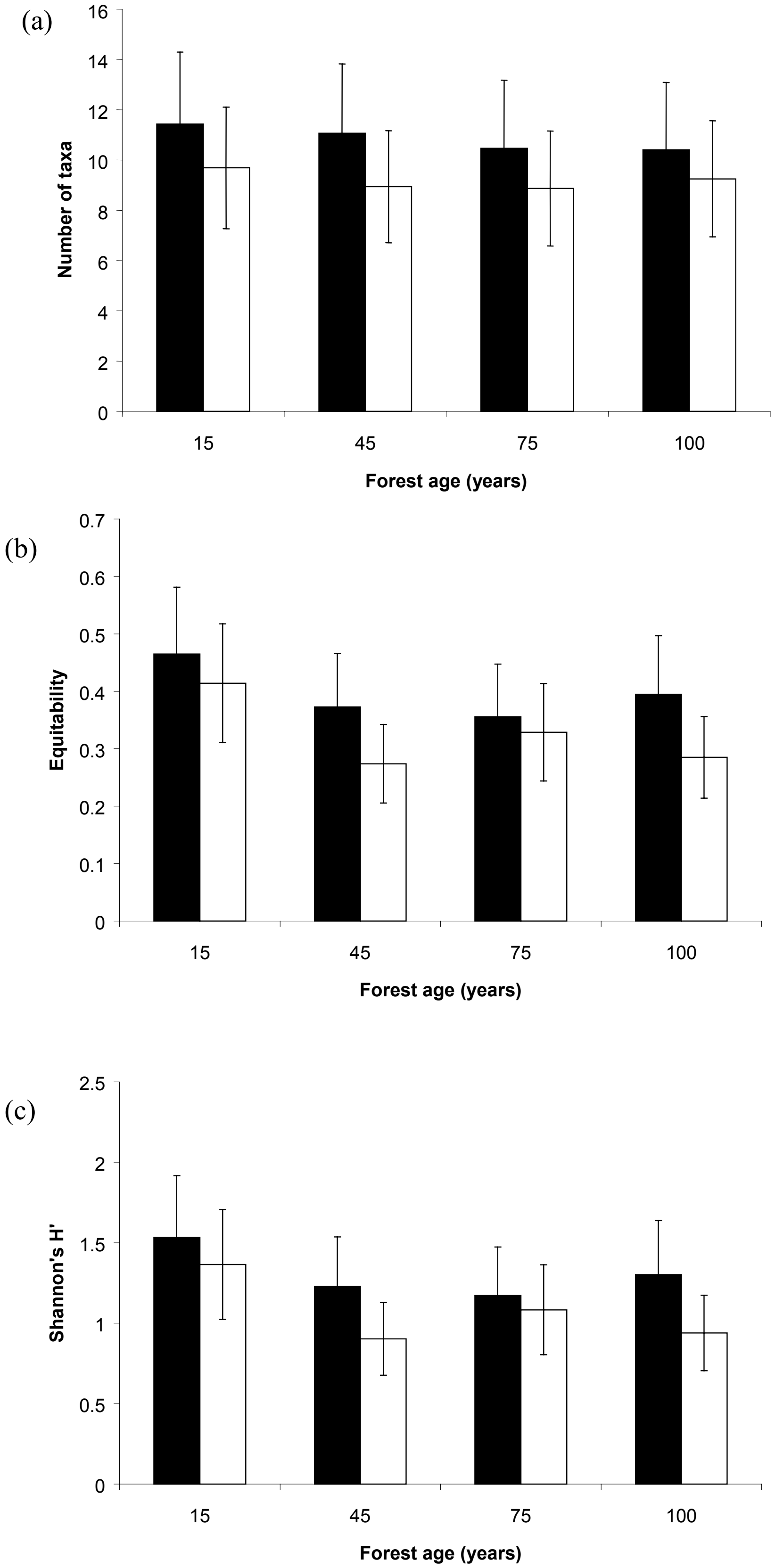


Figure 5.8 (a) Number, (b) equitablility and (c) Shannon's H' of macroinvertebrate taxa present in decomposition boxes placed in four forest successional stages (15, 45, 75 and 100 years of age). Dark bars represent decomposition boxes containing *Persea americana* leaves and light bars boxes with *Pinus chiapensis* needles. All collection dates pooled. Values presented are mean ± standard error. Sample size (n) was 16 per foliar species in each successional stage except for the 75-year-old (*P. americana* and *P. chiapensis*) and the 100-year-old (*P. americana* only) forests where n=15. For all three variables values were significantly higher (ANOVA $P < 0.05$) for *P. americana* boxes than *P. chiapensis* boxes independently of forest age.

Although formal testing of the interaction between successional stage and collection time was not possible with the ANOVA design used in this experiment, in the 15- and 100-year-old forests the equitability and Shannon's indices appear to changes through time while they seem to remain constant in the 45 and 75-year-old forests. In the 15- and 100-year-old forests both indices seem to be initially intermediate (first collection date, after 28 days of box placement), then declined to their lowest value (second collection date, after 56 days) increasing with time thereafter (third and fourth collection dates, after 112 days 220 days; figure 5.7 b and c).

Abundance of individual macroinvertebrate taxa

Of the total number of macroinvertebrates present in experimental decomposition boxes at the time of collection, 82% (27,684) were Collembola. Of the remaining 20% (6081), the most abundant groups were Acari 22.6% (1375), Coleoptera 20% (1214), Diplopoda 12.8% (778) and Diptera larvae 11% (667). The rarest taxa were Uropygi, Thysanura, Blattodea and Diptera (total of 1, 2, 3 and 5 individuals respectively). Dermaptera and Lumbricina were completely absent in the decomposition boxes.

The abundance of macroinvertebrate taxa in the decomposition boxes varied between collection dates. Often the abundance of taxa was lowest at the first two collection dates and increased at the third and fourth. This was the case for Acari, Diplura, Hymenoptera, Enchytraeidae, Gasteropoda and Aranea. In contrast, Lepidoptera larvae were most abundant at the first and second collection dates, while Pseudoescorpionida were most abundant at the fourth and Collembola at the second and third (figure 5.9).

Independent of other treatments, some taxa were consistently more abundant in decomposition boxes recovered from particular successional stages. Hemiptera were most abundant in the 75-year-old forest and Pseudoescorpionida in the 15 and 45-year-old forests. Other taxa displayed relatively low values of abundance at certain successional stages such as Diplopoda and Aranea in the 75-year-old forest or Coleoptera and Collembola in the 15-year-old forest (figure 5.9).

Across the experiment, taxa were often more abundant in boxes containing *Persea americana* leaves than in those containing *Pinus chiapensis* needles. This was the case for Aranea, Hemiptera, Enchytraeidae and Diptera larvae. Only Coleoptera (adults) were systematically more abundant in the *Pinus chiapensis* boxes (figure 5.9).

The abundance of some taxa responded to particular combinations of treatments. In general the abundance of Diplura was relatively high in the 75- and 45-year-old forests and at the last two collection dates. However, the effect of collection date was more pronounced in the boxes containing *Persea americana* leaves (figure 5.9). In the case of Enchytraeidae, abundance was also higher in *Persea americana* boxes and at the last two collection dates. The highest number of Enchytraeidae recorded at the third collection date was in the 45-year-old forest, followed by the 100 and 75-year-old forests. In contrast, at the fourth collection date the abundance was highest in the 15-year-old forest and diminished with forest age (figure 5.9). Coleoptera larvae uniquely had a peak of abundance in the *Persea americana* boxes recovered from the 100-year-old forest at the third collection date.

Several macroinvertebrate taxa did not vary noticeably in their abundance between experimental treatments or the variation amongst replicates was so high that no pattern was detected. These cases included Chilopoda, Orthoptera, Homoptera, Formicidae, Isopoda, and Diptera larvae.

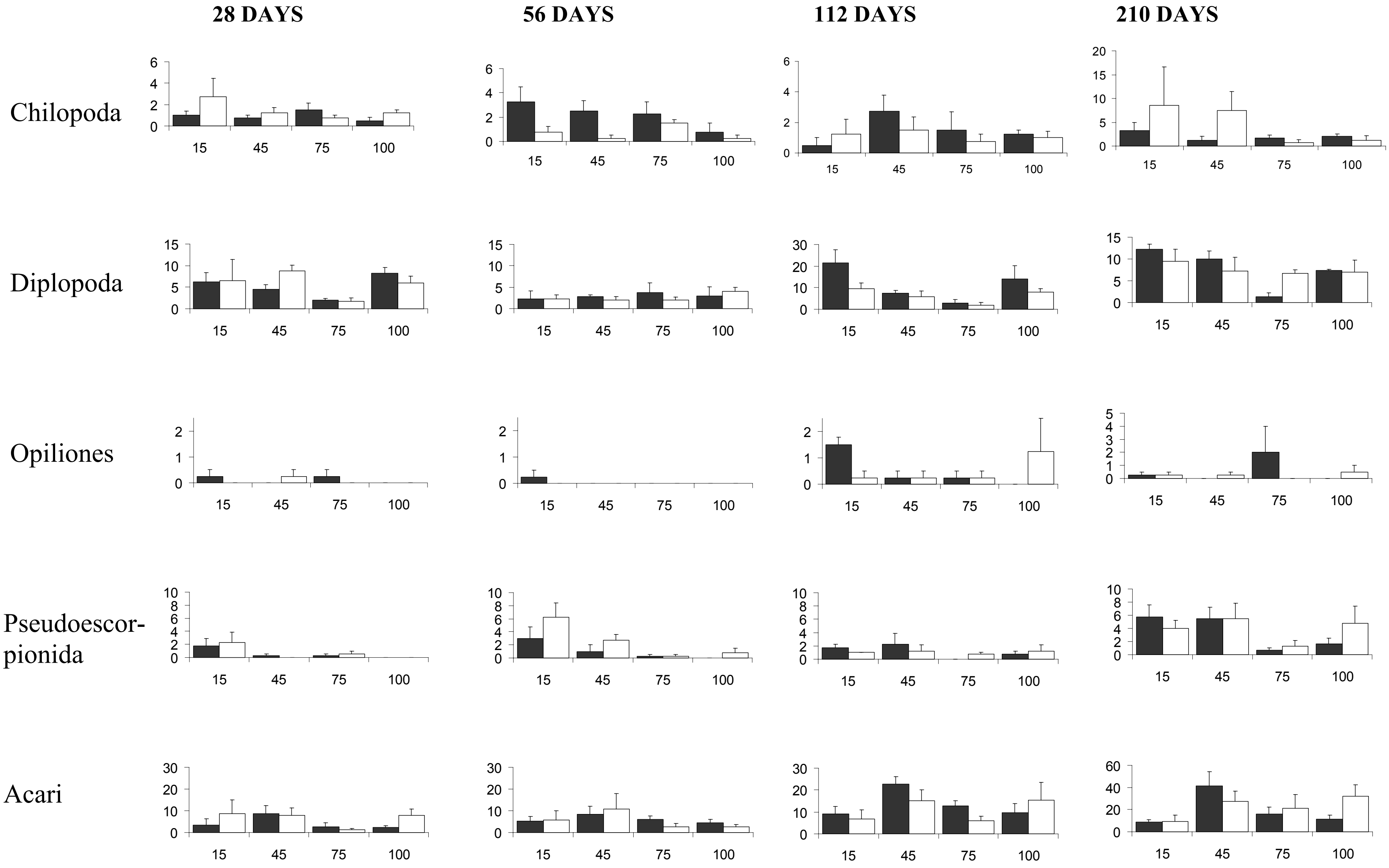
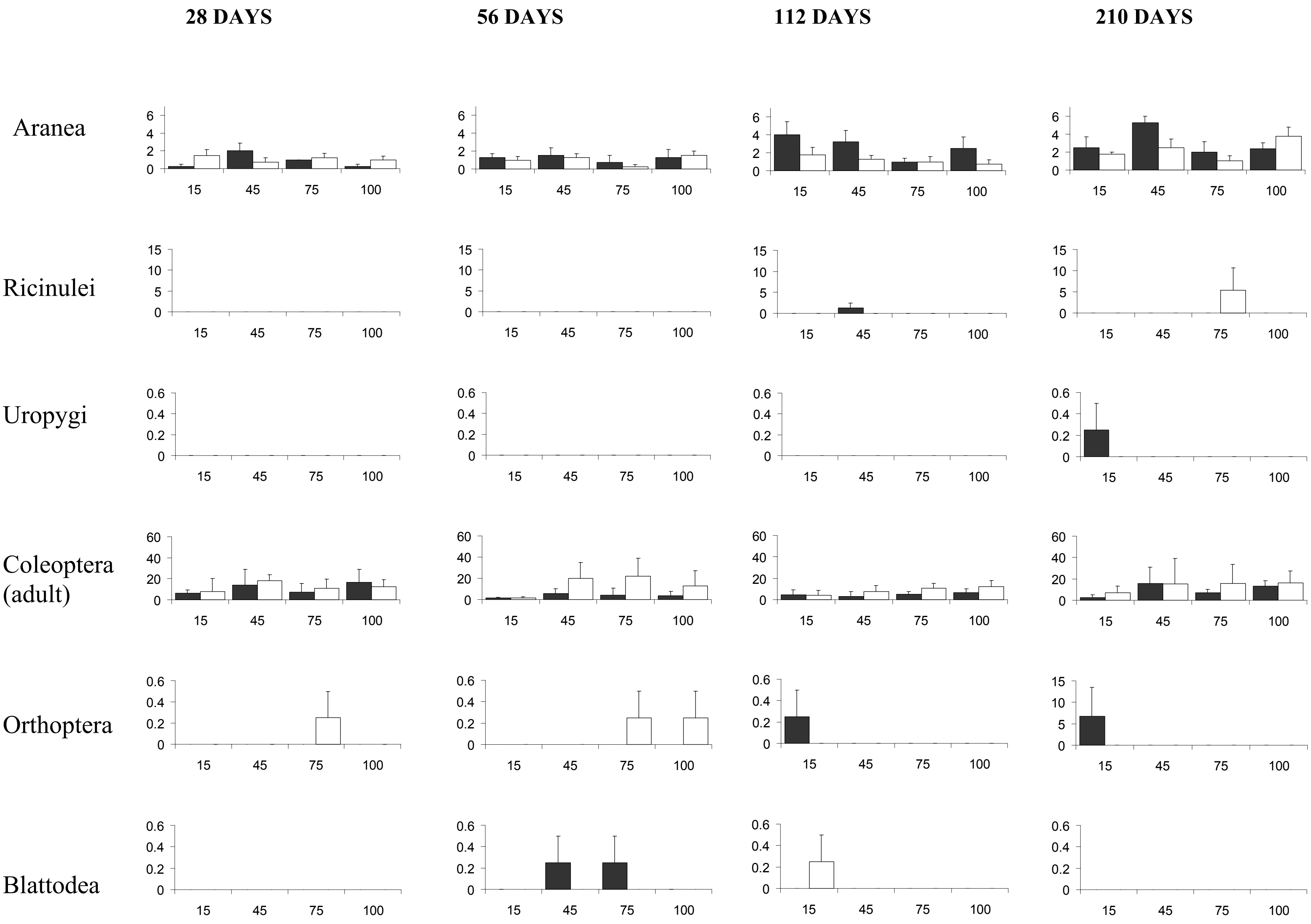


Figure 5.9. Abundance of macroinvertebrate taxa found in experimental decomposition boxes in different successional stages (15, 45, 75 and 100 years old) of the Tarantulas chronosequence. The mean ± standard error of the abundance per successional stage is presented for four collection dates (28, 56, 112 and 210 days after placement). Filled bars represent decomposition boxes containing *Persea americana* leaves and open bars boxes with *Pinus chiapensis* needles. Sample size (n) was four per foliar species in each successional stage, except for the collection after 210 days in the 75-year-old (*P. americana* and *P. chiapensis*) and the 100-year-old (*P. americana* only) forests where n=3. Note changes in scale in the y-axis that have been made to accommodate substantial differences in taxa abundance.



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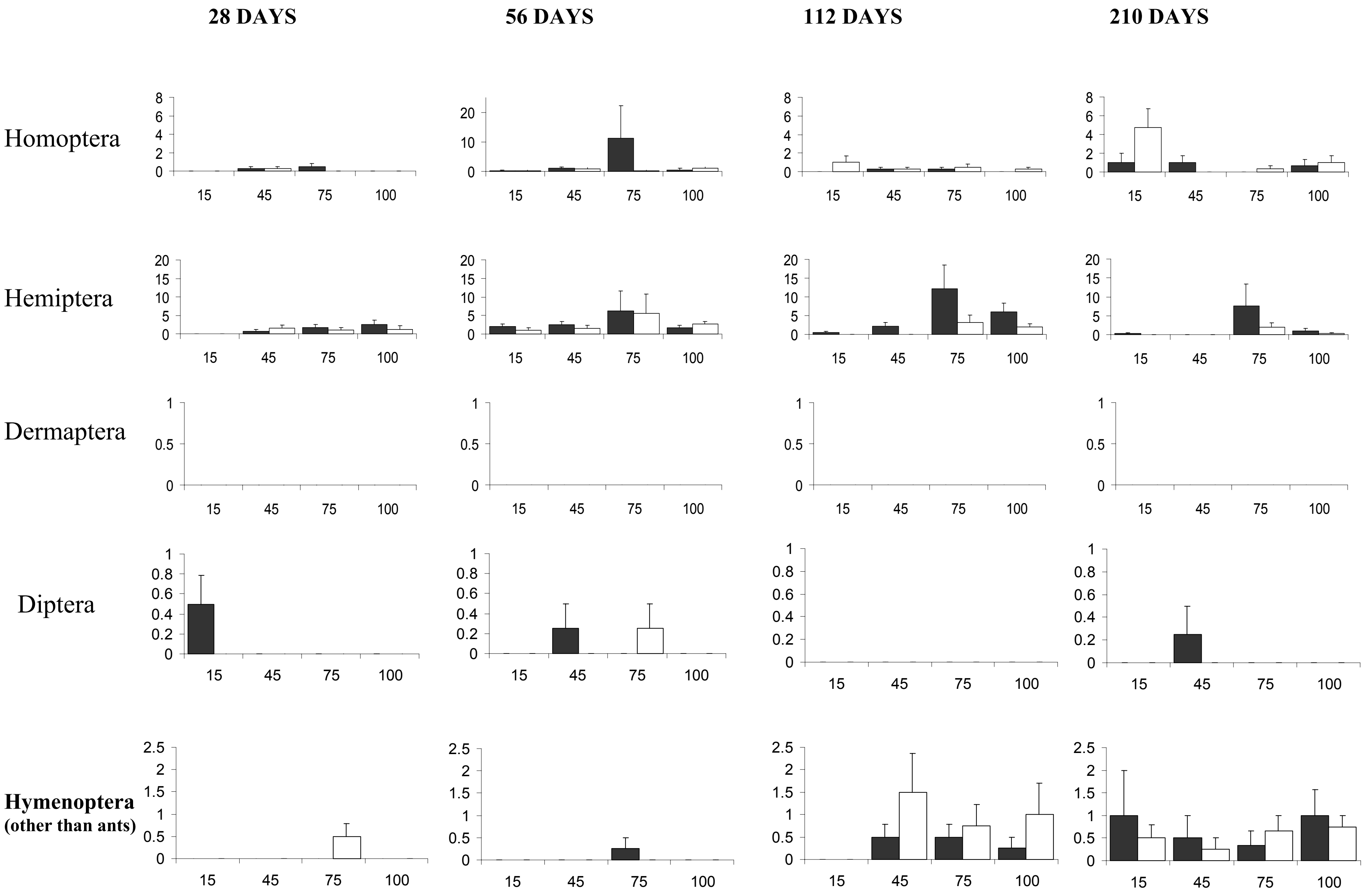

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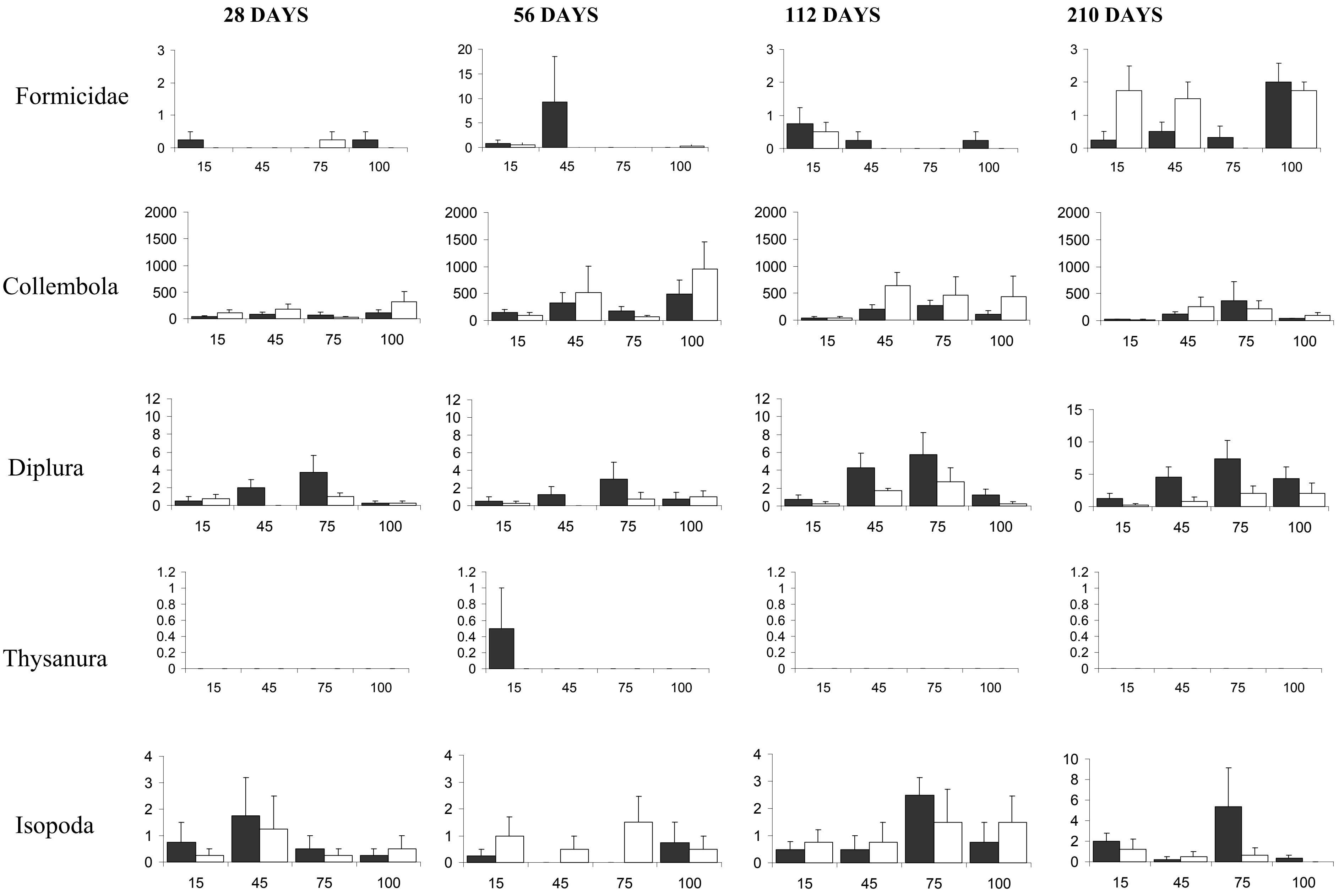

Figure 5.9 continuation...

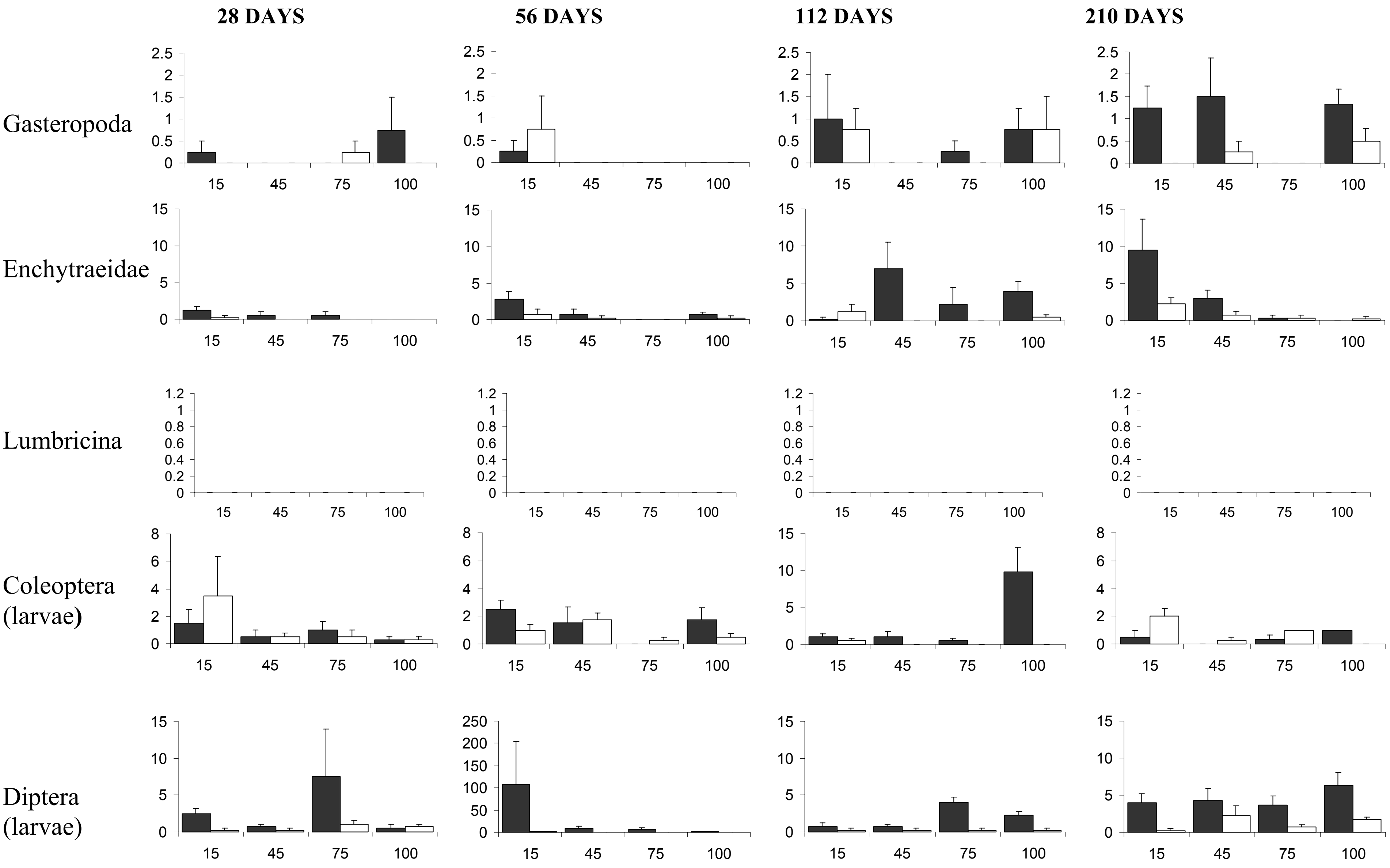

Figure 5.9 continuation...

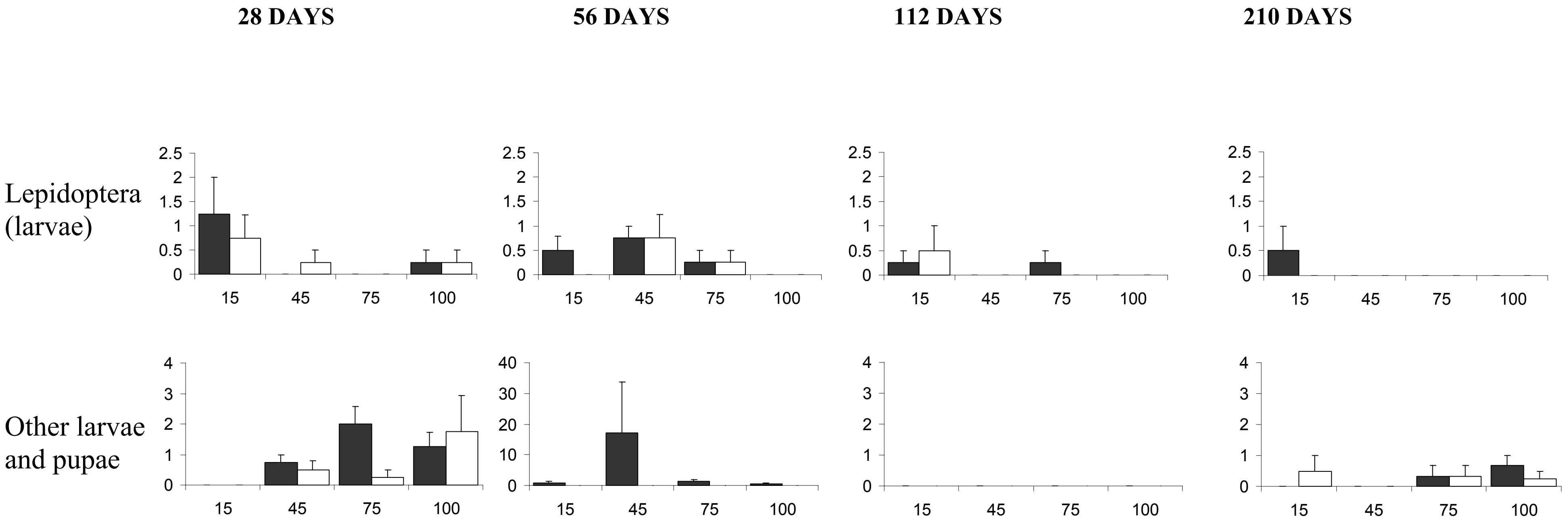

Figure 5.9 continuation...(ends)

Correlation between macroinvertebrate community composition and decomposing leaf species, successional stage and collection date

A canonical correspondence analysis of the macroinvertebrate community in experimental decomposition boxes with respect to experimental treatments and leaf chemistry showed a number of associations between community composition and the combination of successional stage, collection date, leaf species and sample chemical composition. The variables tested explained approximately 21.5% of the total variance (table 5.3). When the variance was partitioned among sets of variables, the concentrations of nutrients, RF and ASF (nutrient chemistry in table 5.3) accounted for the highest amount of explained variation (14.7%), followed by successional stage (7.5%), collection date (4.8%) and leaf species (2.6%).

Groups of explanatory variables included in CCA	**Extracted inertia from a total of 1.952**	**% of taxa variance explained (all axis)**	**Significance of Monte-Carlo test**
Successional stage	0.147	7.5	*
Collection date	0.093	4.8	*
Leaf species	0.05	2.6	*
Nutrient chemistry	0.315	14.7	*
All variables	0.482	21.5	*

Table 5.3 Partitioning of macroinvertebrate taxa total inertia in CCA amongst different groups of explanatory variables. Includes all experimental boxes. Notice that the CCAs were run independently from each other and therefore the sum of the extracted inertia per group of variables does not correspond to the extracted inertia by a single run including all variables. * denotes $P<0.05$ in Monte-Carlo significance tests.

When the decomposition boxes recovered from each successional stage were examined separately (figures 5.10 to 5.12), in the 15-, 45- and 100-year-old forests a significant association was recorded between community composition and collection date, species of leaf being decomposed and nutrient, RF and ASF concentrations (Monte-Carlo $P<0.05$). There was no significant association between variables in the 75-year-old forest.

In the 100-year-old forest, the sets of variables tested explained approximately 43.1% of the total variance (eigenvalues: unconstrained 0.832, canonical 0.411), of which 36% was represented by the first two canonical axes (figure 5.10). In the canonical space of this forest, the samples from decomposition boxes retrieved at the last collection date (after 220 days) form an isolated group. This position denotes a more distinct community composition characterised mainly by Formicidae and Pseudoscorpionidae, and to a lesser extent, Hymenoptera, Acari and other larvae. The communities recorded at this collection were associated with higher concentrations of phosphorous in the decomposing leaves. A second group of points identifiable in the canonical space was associated with higher concentration of magnesium, calcium, RF and ASF as well as lower concentrations of potassium and total carbon. The group was formed principally by the samples recovered after 112 days and its communities were characterised by the presence of Coleoptera larvae, Enchytraeidae, Hemiptera, Phalangida and Isopoda as distinctive elements. In this ordination of the samples retrieved from the 100-year-old forest, the species of leaves being decomposed and the concentration of nitrogen did not play a significant role in explaining the variance in community composition. With respect to the samples retrieved at the first two collection dates (after 28 and 56 days), leaf species and chemistry did not explain the variance of taxa to a significant degree.

In the decomposition boxes retrieved from the 45-year-old forest, 57.8% of the variance (eigenvalues: unconstrained 1.204, canonical 0.745) was explained by experimental treatments and the chemical composition of leaves. The first two canonical axes represented 51.6% of the variance (figure 5.11), but the first canonical axis alone was not significant. In the canonical space of this successional stage the community composition was explained principally by the concentration of nutrients, but these were not associated with any specific collection date. Decomposition boxes that contained *Persea americana* leaves displayed higher concentrations of calcium, magnesium, sodium, phosphorus and ASF and lower concentrations of RF and total carbon. These samples were associated with Diptera, Coleoptera and Lepidoptera larvae and to a lesser extent Enchytraeidae and Diptera.

In the experimental boxes placed in the 15-year-old forest, 51.8% of the variance (eigenvalues: unconstrained 1.791, canonical 1.052) in community data was explained by collection date, species of leaf and chemical concentration variables.

The first two canonical axes represented 42.6% of the variance (figure 5.12). In the ordination space of the 15-year-old forest, samples collected at the first two collection dates (after 28 and 56 days) had higher concentrations of sodium and potassium. The communities in these samples were characterised by Hemiptera, Collembola and Diptera. The leaves in experimental boxes collected after 112 days had higher concentrations of RF and lower concentrations of potassium. The communities in these boxes had a diverse community that included several groups such as Chilopoda, Acari, Phalangida, Diplura, Gasteropoda, Isopoda, Formicidae, Pseudoescorpionida, Coleoptera, Aranea and Diplopoda. The boxes collected at the fourth collection date (after 220 days) had a higher concentration of phosphorus and a lower potassium concentration. In these boxes, the community composition was similar to that of the previous collection date, but in this case Hymenoptera, Enchytraeidae and Homoptera where particularly frequent. In this ordination the species of leaf being decomposed did not play a significant role in explaining variance in the community composition.

When the communities recovered at each collection date were analysed separately, the composition of the communities in experimental boxes retrieved after 112 days and 220 days were significantly associated with successional stage and chemical composition of the sample at the time of retrieval (Monte-Carlo $P<0.05$). For the boxes collected after 112 days 46.9% of taxa variance was explained (eigenvalues: unconstrained 0.690, canonical 0.380) and for the boxes collected after 220 days 45.7% of taxa variance was explained (eigenvalues: unconstrained 1.149, canonical 0.604). In contrast, there was no significant association between community composition and these variables at the first two collection dates (after 28 and 56 days). In the canonical spaces of both, the samples recovered after 112 days and those recovered after 220 days, the most isolated group of points was that formed by the 15-year-old forest samples (figures 5.13b and 5.14b). In the case of the 112 days collection date these samples were associated with low concentrations of nitrogen and had a distinctive community composition with Orthoptera, Opiliones, Formicidae, Gasteropoda and Lepidoptera larvae as distinctive elements (figure 5.13a). To a lesser extent, these communities were also characterised by the presence

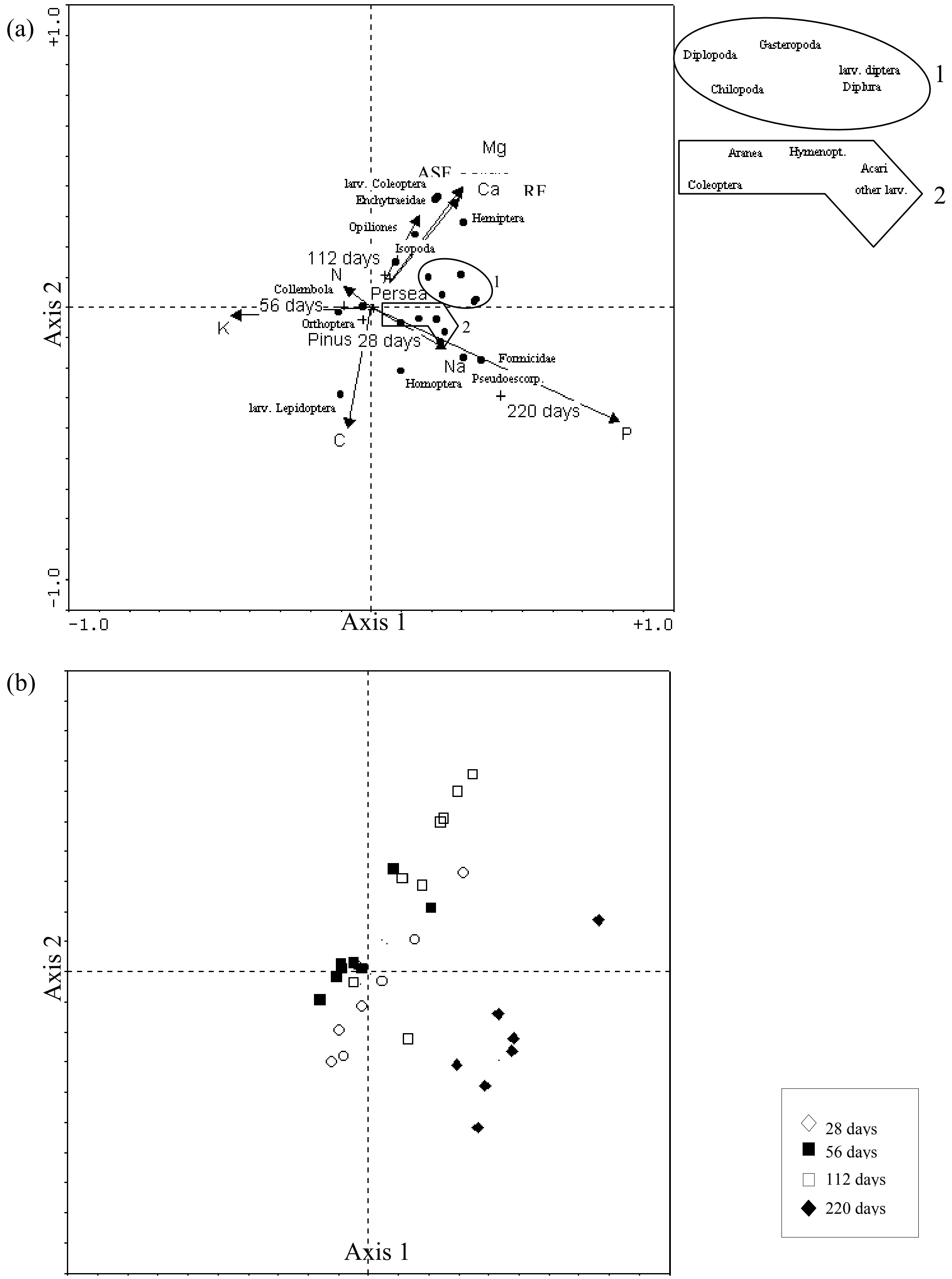

Figure 5.10 Ordination diagrams based on canonical correspondence analyses of community composition in the decomposition boxes placed in the 100-year-old forest. Community composition is ordinated with respect to number of days in the field (dummy variables), species of leaf decomposed in the box (dummy variables) and nutrient, ASF and RF concentration at the time of collection. The first two axes account for 36.5% of variation, Monte-Carlo permutations significant test $p<0.05$ (taxa with fewer than five individuals in the experiment are excluded). (a) Biplot of taxa-explanatory variables and (b) sample scatter plots symbol coded by collection date. For clarity, insets in (a) provides labels for the taxa points within the ellipse and polygon denoted 1 and 2.

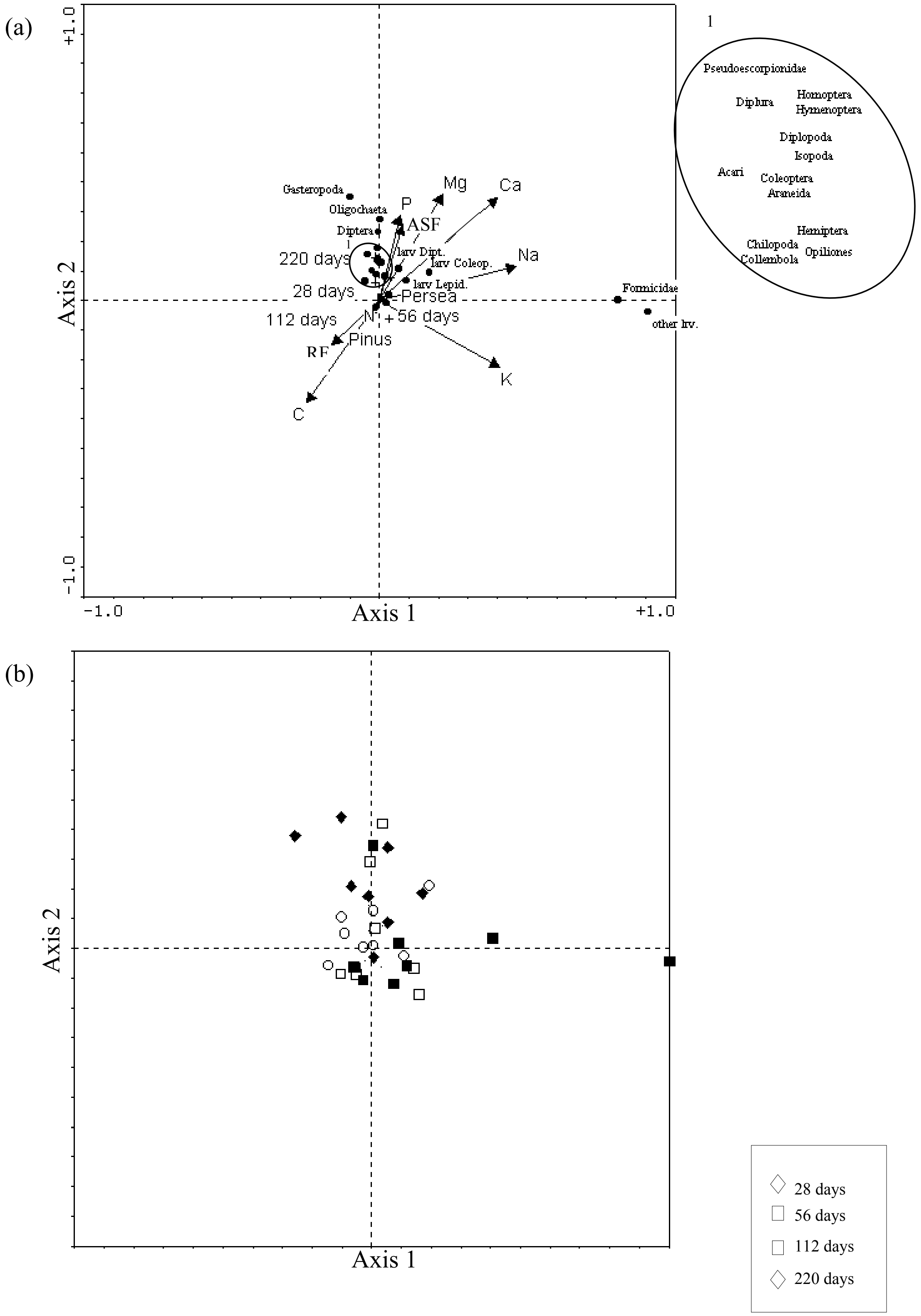

Figure 5.11 Ordination diagrams based on canonical correspondence analyses of community composition in the decomposition boxes placed in the 45-year-old forest. Community composition is ordinated with respect to number of days in the field (dummy variables), species of leaf decomposed in the box (dummy variables) and nutrient, ASF and RF concentration at the time of collection. The first two axes account for 51.6% of variation, Monte-Carlo permutations significant test $p<0.04$ (Taxa with fewer than five individuals in the experiment are excluded). (a) Biplot of taxa-explanatory variables and (b) sample scatter plots symbol coded by collection date. For clarity, inset in (a) provides labels for the taxa points within the ellipse denoted 1.

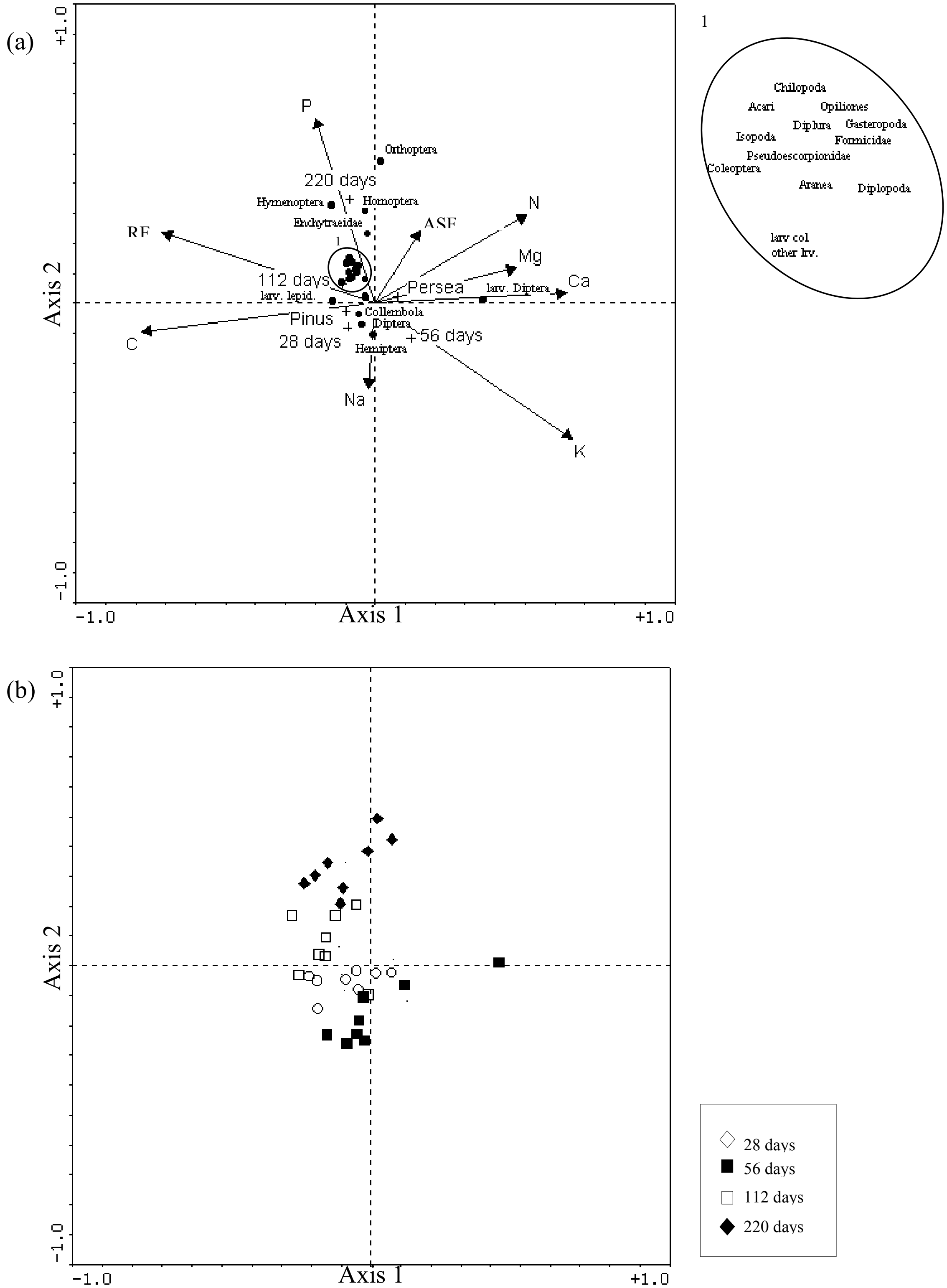

Figure 5.12 Ordination diagrams based on canonical correspondence analyses of community composition in the decomposition boxes placed in the 15-year-old forest. Community composition is ordinated with respect to number of days in the field (dummy variables), species of leaf decomposed in the box (dummy variables) and nutrient, ASF and RF concentration at the time of collection. The first two axes account for 42.6% of variation, Monte-Carlo permutations significant test $p<0.005$ (taxa with fewer than five individuals in the experiment are excluded). (a) Biplot of taxa-explanatory variables and (b) sample scatterplots symbol coded by collection date. For clarity, inset in (a) provides labels for the taxa points within the ellipse denoted 1.

of Homoptera, Aranea, Diplopoda and Pseudoescorpionida. At the 220 days collection date, the samples extracted from the 15-year-old forest had higher concentrations of calcium and RF as well as lower concentrations of phosphorus and total carbon. The macroinvertebrate communities in these boxes had Orthoptera, Enchytraeidae, Lepidoptera larvae, Homoptera and Coleoptera larvae as distinctive elements (figure 5.14a).

In ordination space of the samples collected after 112 days the samples recovered from the 45-, 75- and 100-year-old forests formed a combined group of points spread along a gradient of increasing concentrations calcium, magnesium and ASF together with decreasing potassium and total carbon. In this group, samples with higher concentrations of calcium, magnesium and ASF were characterised by higher numbers of Hemiptera, Lepidoptera larvae, Coleoptera larvae, Enchytraeidae and Diplura (figure 5.13). Similarly, in the decomposition boxes collected after 220 days, the samples recovered from the 45-, 75- and 100-year-old forests formed a combined group of points spread along a gradient of increasing sodium concentration together with decreasing potassium and magnesium. However, the community composition of these samples had fewer distinctive elements associated with the changes in chemistry of the leaves. Only abundance of Diptera appeared to be related to lower concentrations of potassium and magnesium, whereas abundance of Opiliones was characteristic of higher concentrations of these elements (figure 5.14).

Discussion

The relationship between succession and decomposition rate

In general, decomposing litter is formed by a labile and a resistant component. The first decays within one year of decomposition and accounts for about 70% of the litter mass. The second, which is formed by the least labile molecules such as lignin, decomposes in about 10 years and accounts for circa 30% of the litter mass. For this reason simple negative exponential models are a good model of the first year of decomposition but only a crude approximation to the full course of litter decay. A double exponential equation is indeed a better approximation for modelling long

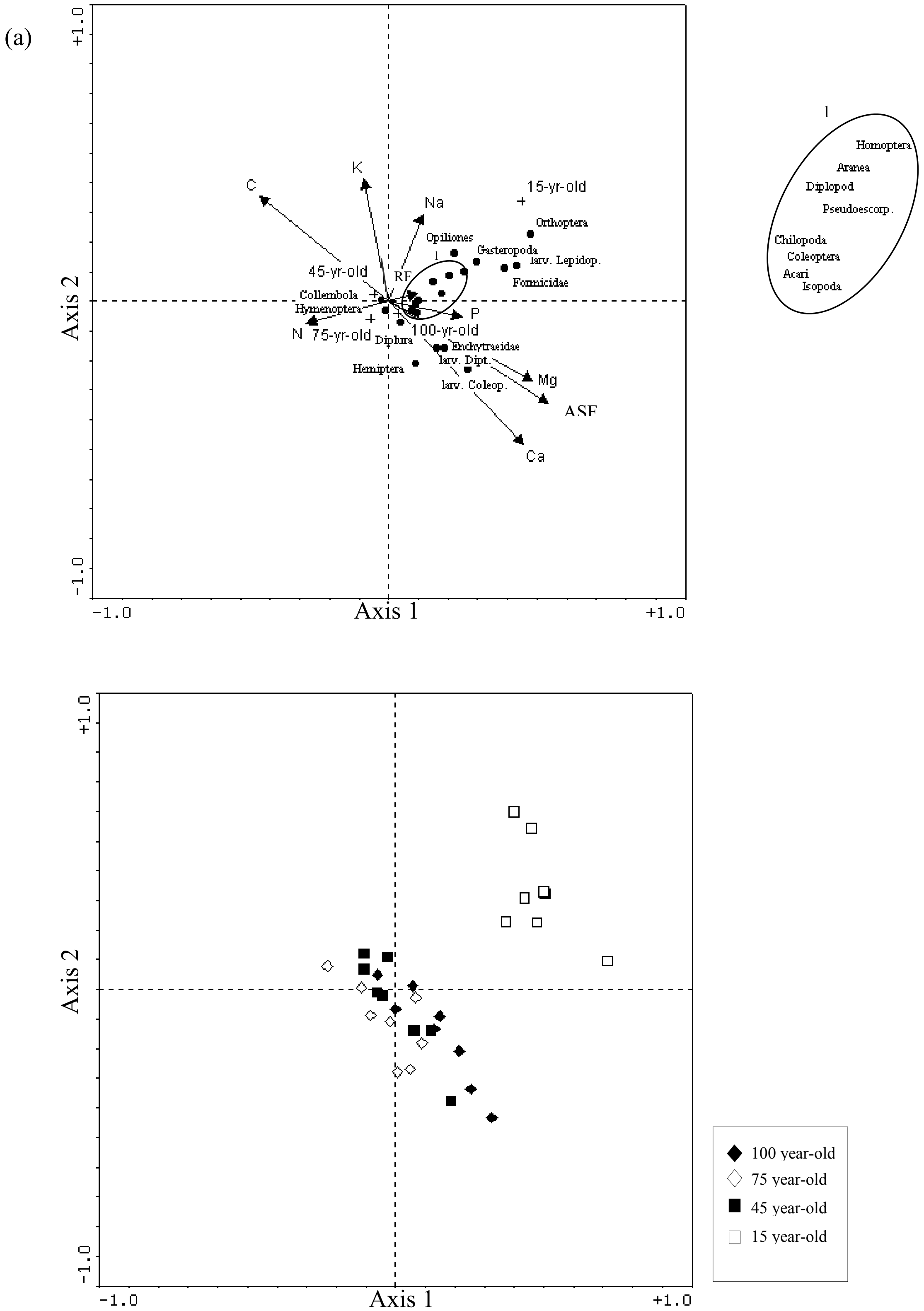

Figure 5.13 Ordination diagrams based on canonical correspondence analyses of community composition in the decomposition boxes after 112 days after the start of the experiment. Community composition is ordinated with respect to successional stage (dummy variables) and nutrient, ASF and RF concentration at the time of collection. The first two axes account for 38.5% of variation, Monte-Carlo permutations significant test $p<0.005$ (taxa with fewer than five individuals in the experiment are excluded). (a) Biplot of taxa-explanatory variables and (b) sample scatter plots symbol coded by successional stage. For clarity, inset in (a) provides labels for the taxa points within the ellipse denoted 1.

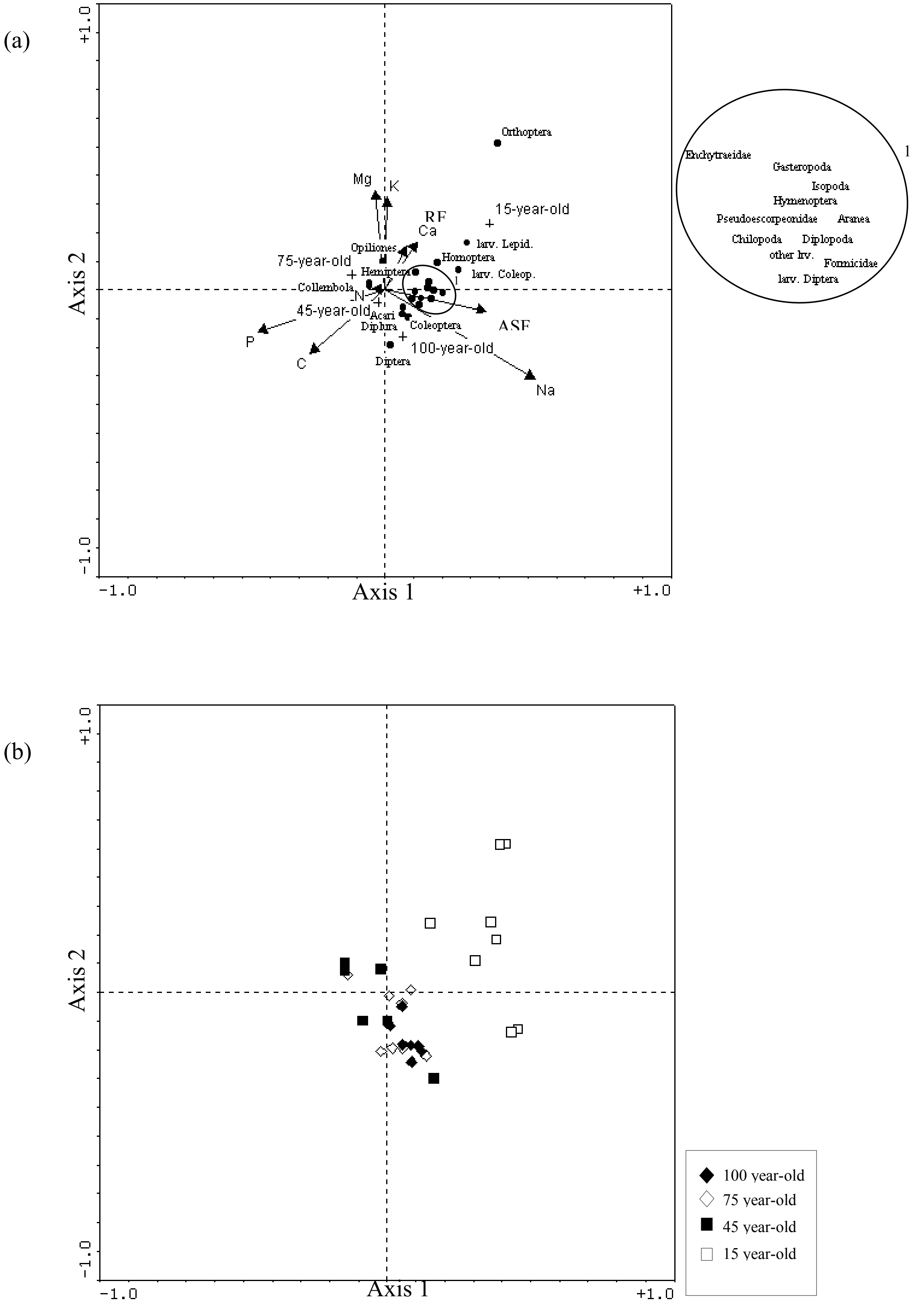

Figure 5.14 Ordination diagrams based on canonical correspondence analyses of community composition in the decomposition boxes after 220 days after the start of the experiment. Community composition is ordinated with respect to successional stage (dummy variables) and nutrient, ASF and RF concentration at the time of collection. The first two axes account for 35.2% of variation, Monte-Carlo permutations significant test $p<0.01$ (taxa with fewer than five individuals in the experiment are excluded). (a) Biplot of taxa-explanatory variables and (b) sample scatter plots symbol coded by successional stage.

term decay and there are data for the full course of decomposition (Swift *et al.* 1979). In this thesis it was found that about 60% of the litter mass would have disappeared at the end of the first year of decomposition, while the remaining 40% was resistant to decay in this initial phase. It is possible that the cool conditions combined with the low litter quality prevailing in TMCF resulted in a slower initial decomposition phase than found for other ecosystems (see below).

Decomposition rate in high altitude TMCF is low compared to forests at lower altitudes and is thought to be primarily limited by microenvironmental conditions, namely low temperatures (Vitousek *et al.*, 1994; Scowcroft *et al.*, 2000). The decomposition rate in Tarantulas was low as in other TMCF of similar altitude. The first-order decay exponential constants (k) of both species in the experiment were within the range of those found for several species in other studies (k= 0.2-1.3) (Tanner, 1981; Vitousek *et al.*, 1994; Crews *et al.*, 1995; Scowcroft *et al.*, 2000). The decomposition rate of *Persea alpigena* is the only value reported in the literature for TMCF that is directly comparable to the results obtained here, given that a congeneric species was investigated. From the results presented here, after a year of decomposition it is predicted that 38% of the initial *P. americana* leaf mass would remain (extrapolating from an exponential decay with a k= 0.97) which is very similar to the remaining mass of *P. alpigena* (37%) recorded after one year of decomposition in a Jamaican montane forest (Tanner, 1981).

There was no significant difference in the decomposition rate between successional stages. This result is consistent with the hypothesis that decomposition rate is primarily constrained by climatic factors imposed by constant fog cover (Bruijnzeel & Veneklaas, 1998). Even if there were substantial differences in the soil nutrient availability between successional stages, full canopy cover is already present in the 15-year-old forest and the maximum soil temperature difference was only *c*. 1ºC between the earliest and latest successional stages (see Chapter 3). This suggests that after about 15 years of succession, fog retention capacity of the forest is developed enough (although not fully, given small differences in soil temperature) to constrain decomposition rate.

The exact magnitude of the effect of increasing temperature on decomposition rate in tropical montane forests is still somewhat unclear. This issue has been primarily studied through reciprocal transplant and common-garden experiments across elevation gradients in Hawaiian tropical montane forests. A wide range of Q_{10} values for decomposition (1.5-6.2 factor of rate increase per every 10ºC increase in environmental temperature) has been recorded (Vitousek *et al.*, 1994; Scowcroft *et al.*, 2000). These studies acknowledge the prime importance of temperature as a litter decomposition driver in these forests; however, they also recognise that the extent of its effect can be accentuated or counteracted by other factors that co-vary with elevation, such as site age, soil fertility, humidity and litter quality. With the data available in this study, it is only possible to speculate that the decomposition rate in all of the Tarantulas chronosequence may be intermediate between recently logged sites, where canopy has been recently opened and mean soil temperature is *c*.2ºC higher, and pristine sites where canopy structure is most complex and soil temperature is *c*. 2ºC lower (see Chapter 3).

If climatic differences do not reduce decomposition rate during succession between 15 and 100 years after logging, why is organic matter accumulating and nutrient availability declining? In the second half of the experiment, mass loss in *P. americana* was lower than in *P. chiapensis* in all successional stages. Extrapolating from the exponential decay models, after one year, *P. chiapensis* would have lost 7% more mass than *P. americana*. If decomposition rates of most late successional species were similar to that of *P. americana* (this will be further explored in Chapter 6) this difference in decomposition rate could account for at least a portion of the accumulated organic matter with succession. However, the fact that considerably more standing litter crop accumulates in early-intermediate than in later stages of succession seems contradictory to this finding. A possible explanation is that decomposition rate is primarily restricted by climatic factors in all successional stages as proposed above. Although the rate of decomposition of late successional stress-tolerant trees (such as *P. americana*) may be slightly lower than early successional species, those holarctic species dominating in early-mid succession produce more abundant litter (Williams-Linera & Toledo, 1996; see Chapter 1) which, given the climatic constraints, accumulates even if it decomposes slightly more rapidly. Furthermore, it is possible that the accumulated organic matter in late-successional soils is largely a result of the combination of a low decomposition rate and abundant litter production by pioneer trees during earlier succession. After logging, during the time when the canopy is open (c. 15 years) the in-put of high quality organic matter combined with higher irradiance penetrating the canopy may increase decomposition rate and render the soil more fertile (as shown in Chapter 1). However, as soon as the canopy closes, decomposition rate probably declines again, as seems to be the case in the study by Addison *et al.* (2003) who found that decomposition rate of pine needles in coastal temperate forests (Vancouver Island) was more rapid in recently cleared sites (7-9 year-old) but found no differences between older sites (35-46 year old, 80-102 year old and >248 years).

In summary, the evidence shown here does not support the hypothesis that decomposition rate declines as succession proceeds between 15 and 100 years after logging. I suggest that this is because climatic constraints imposed by frequent fog have already developed after 15 years of succession and that the accumulation of organic matter and the nutrient poor soils develop as the relatively fast-growing vegetation depletes soil of the nutrients that became available as a result of the opening of the canopy.

Litter quality and decomposition rate

The moderately lower rates of mass loss of *P. americana* compared to that of *P. chiapensis* could be interpreted as an indication of differences in litter quality (Swift *et al.*, 1979). The initial quality of the leaves in these two species differed little, particularly in terms of those aspects that have previously been shown as good predictors of decomposition rate, such as nitrogen, phosphorous, and fibre (RF and ASF) concentrations (see for example Tanner, 1981; Berg, 1986 and Vitousek *et al.*, 1994). Surprisingly, *P. americana* decomposed relatively slowly, and had a slightly lower initial carbon concentration, and higher sodium, potassium and magnesium concentrations. Furthermore, *P. chiapensis* immobilised N and P in most successional stages while *P. americana* did not undergo net immobilisation of N at any stage and it mineralised P more frequently than *P. chiapensis*. The key to the difference in mass loss between the two species may be in the trend of accumulation of recalcitrant material through decomposition. This was revealed by the evolution of the PCA2 foliar quality component whose increase represented the concentration of Acid Detergent Lignin (referred to as RF) and nitrogen. In the second half of the experiment, when *P. americana* started loosing comparatively less mass, PCA2 was significantly higher than in the first half, while for *P. chiapensis*, this foliar quality component did not vary significantly across the experiment. Similar results have been observed in other cases suggesting that where both N and lignin are present at high concentrations in leaves, lignin exerts a greater control over decomposition (White *et al.*, 1988). Although initially the concentrations extracted from plant litter through the consecutive solutions in acid (NDF, ADF, LDF) represent roughly hemicellulose, cellulose and lignin fractions, during the process of decomposition other recalcitrant (humic) materials are synthesised and these are extracted as part of the ADF and LDF fractions (McClaugherty *et al.*, 1985). The fact that the amount of RF became rapidly more than 100 % of the initial net content, together with the evidence of significantly different trends of PCA2 between the two species, indicate that during the process of decomposition recalcitrant molecules were being synthesised in both species, but at a higher rate in *P. americana* during the second half of the experiment. The increased accumulation of recalcitrant materials in *P. americana* may account for its relatively low decomposition rate, despite its apparent higher quality indicated by the low N immobilisation and high P mineralisation. The inadequacy of initial nitrogen, phosphorous and lignin concentrations as predictors of mass loss in the leaves of certain species has been pointed out previously by Sanger *et al.* (1998) and they suggest other substances such soluble carbohydrates as alternatives.

It is important to consider the fact that the leaves used for decomposition in this experiment were fresh leaves rather than abscised leaves. Because plants retrieve considerable amounts of nutrients before abscission (Sanger *et al.*, 1998), the results presented here should be interpreted with caution when analysing the biogeochemical cycle of the chronosequence. In Chapter 6 I present the nutrient, RF and ASF contents of freshly abscised leaves of *P. chiapensis* and *Beilschmiedia ovalis* (similar to *P. americana* in that it is a late-successional tree of the Lauraceae family), which can serve for comparison (see tables 5.1 and 6.5). The nutrient concentration in the fresh and abscised needles of *P. chiapensis* were surprisingly similar, except for P concentration that was 19 % higher in abscised needles and K and Mg concentrations that were 190% and 40% higher in fresh needles. All other nutrients were very similar in concentration. ASF and RF concentrations were 25% and 14% higher in the abscised needles than in fresh needles. When comparing fresh *P. americana* with abscised *B. ovalis* leaves, larger differences were found. All of the nutrients concentrations were considerably higher in fresh *P. americana* leaves (N:75%, P:73%, Na:37%, K:5.1%, Ca:2.5%, Mg:1.7% higher). ASF and RF concentrations were 72% and 63% higher in abscised *B. ovalis*. Although with the data available it is not possible to determine how much of the differences found between the Lauraceae members is explainable by species identity and how much by chemical changes during senescence, it can be concluded that fresh and abscised *P. chiapensis* needles have fairly similar chemical composition.

In terms of the decomposition rate, in Chapter 6 I will show that abscised *B. ovalis* leaves decompose significantly more slowly than *P. chiapensis* needles and therefore the decomposition trends observed in this chapter can be extrapolated to abscised leaves with more confidence.

Even if there is some evidence from other studies that less nutrients are mineralised in pine needles than in broad-leaves (Satti *et al.*, 2003), extreme caution should be taken when interpreting the results of the chemical evolution in this experiment. *P. americana* leaves may have had significantly higher quality in terms of nutrient and structural molecule concentrations, and therefore probably mineralised substantially more nutrients than expected for recently abscised leaves from the same species, particularly with respect to P. Abscised leaves from two late-successional species were shown in Chapter 1 to have lower concentrations of P than *P. chiapensis*. Because P concentrations seem to be very low in the soils across the whole chronosequence (see Chapter 3), it is likely that *P. americana* and other late-successional broad-leaved tree species have an efficient reabsorption mechanism for this element during senescence and therefore the leaves used in this experiment do not reflect the patterns naturally occurring in abscised leaves in late succession. However, for *P. chiapensis* the concentrations of nutrients between abscised and fresh needles were fairly similar and results regarding this species and early-mid successional stages, where its litter dominates, can be interpreted with more confidence.

With the above considerations in mind, it is possible to conclude that even if pine litter dominating early-mid successional stages has a higher decomposition rate than the leaves from late-successional plants, its standing crop and immobilisation of nitrogen and phosphorous are both high. This pattern may be contributing to the conservation of nutrients in early-mid succession by promoting their sequestration in semi-decomposed organic matter down the soil profile. A similar trend was suggested by White *et al.* (1988) for a south Appalachian forest where black locust (a N-fixing shrub) dominates early succession. The low decomposition rate of the lignified leaves of this shrub was thought to contribute to the immobilisation and preservation of N in the abundant soil organic matter of late successional forests.

Soil nutrient availability and litter nutrient release during decomposition

It was hypothesised that the decomposition and nutrient mineralisation rates could be partly driven by differences in the availability of nutrients in the soils of different successional stages. The availability of nutrients in the soil has been shown in several studies to have a significant effect on the decomposition rate and nutrient mineralisation of litter (Crews *et al.*, 1995; Scowcroft *et al.*, 2000; Sariyildiz & Anderson, 2003). As discussed previously, results of this study do not support the part of this hypothesis regarding decomposition rate, and I have suggested that mass loss from litter is primarily constrained by climatic variables dominating all successional stages equally. However, nutrient evolution patterns provide some support for the nutrient mineralisation aspect of this hypothesis. The 15-year-old forest, where the most nutrient-rich soils were found, was the only successional stage where N did not undergo immobilisation in *P. chiapensis* leaves and *P. americana* leaves mineralised more P by the end of the experiment. These results suggest that the interaction between site characteristics and litter quality can determine the release of P and N in different successional stages. The design of the experiment does not allow the effects of soil nutrient availability and climatic factors to be differentiated because they both co-vary with succession. However, owing to the reduced gradient observed in climatic factors across the chronosequence, it is likely that higher nutrient availability in early succession plays a more important role in promoting P and N mineralisation than climate. These results agree with those found in a Hawaiian TMCF where N and P are more likely to be mineralised from litter decaying in sites with high fertility (Crews *et al.*, 1995; Scowcroft *et al.*, 2000).

The successional stage where leaves decomposed did not have any effect on the evolution of cation concentration through decomposition in *P. chiapensis* needles. In contrast, differences were recorded in the concentrations of Na, K and Ca (foliar quality component PCA1) between *P. americana* leaves decomposed in mid-succession and the ones decomposed in other successional stages. Na and K are highly mobile elements, microbial activities are not required for their release and the amount of these elements entering the litter in rainfall and through-fall is considerable (Seastedt, 1984; Hölscher *et al.*, 2003). It is possible that the observed differences between successional stages in the litter content of K and Na are a consequence of differences in the through-fall caused by variation in canopy structure (Hölscher *et al.*, 2003). It is difficult to explain why these elements evolved differently in the two foliar species, but is plausible that differences in initial concentrations and the physical configuration of the leaves make them more or less susceptible to the leaching of these elements.

In Chapter 3 it was shown that the availability of Ca in the soil decreased significantly with succession up to the 75-year-old forest. Even if not statistically significant, the 100-year-old forest had a slightly higher mean of Ca availability than the 75-year-old. In consequence, one would expect the release of this element from decomposing litter to decline with succession as a result of decomposers being increasingly Ca limited up to the 75-year-old forest, and then remain constant or speed-up thereafter. The mean concentration of Ca in decomposing *P. americana* followed the expected pattern. It increased with the age of the forest up to the 75-year-old forest and then diminished in the 100-year-old forest. An important question is why was this not the case for the release of Ca from *P. chiapensis* needles? The reason may lie in the fact that through-out the experiment the concentration of Ca in *P. chiapensis* was substantially lower (16.20 cmol·Kg^{-1}) than in *P. americana* (54.56 cmol·Kg^{-1}). This may imply that there is a minimum Ca concentration in decomposing litter required for the higher availability of the same element in the soil to have an effect on the rate of release. This explanation is supported by the fact that at the first two collection dates (except in the 75-year-old forest) the percent of initial amount of Ca remaining in litter was always higher in *P. chiapensis* than in *P. americana*.

The patterns of mineralisation and immobilisation of P and N observed in the 15-year-old forest, together with the trends in release of Ca from experimental litter, support the hypothesis that the higher availability of nutrients in early succession has a positive effect on the release of these elements from decomposing litter. These results are consistent with the pattern described by Wardle *et al.* (1997a) for a chronosequence of primary succession after fire caused by lightning in conifer boreal forests in Sweden. They showed that N mineralisation (as well as decomposition rate) diminishes with succession. This decline in nutrient release is associated with a decrease in litter fall and litter quality in later successional stages. Findings in this study also agree with the more general suggestion that rapid mineralisation in relatively fertile soils constitutes an important positive feedback mechanism to sustain nutrient availability (Hobbie, 1992; Scowcroft *et al.*, 2000). But then, why is pine litter immobilising N and P from the 45-year-old forest onwards? If in the 15-year-old forest there seems to be a positive feedback that allows nutrients to be released more readily from decomposing litter, then litter quality should be high and stay high. What happens through succession that reverses this mechanism?

The answer may lie in the allocation of resources by early-successional plants. Although abscised pine litter had a higher concentration of P than other foliar species, all species were relatively nutrient-poor compared to other studies in similar forests (see Chapter 3). Pine was lower than late-successional species in terms of cation concentraton even though these were generally available in early succession. This may indicate that pine trees in early succession are not returning an important proportion of the absorbed nutrients to the soil, possibly by allocating resources to growth. This is consistent with the above suggestion that early successional communities may be slowly depleting the resources that became available in the soil as a result of the canopy opening. By the 45-year-old forest, the resources available in the soil may no longer be sufficient for the poor quality litter to mineralise nutrients early in decomposition and they start to be accumulated through immobilisation.

The response of the macroinvertebrate community to experimental leaves in different successional stages

One of the interesting questions that emerged from results in Chapter 3 was why does the richness of soil macroinvertebrate community increase with succession when the standing crop of litter diminishes and the soil becomes increasingly nutrient-poor? This question becomes more intriguing with the finding that N, P and Ca are likely to be released sooner from decomposing litter in early succession.

Even though there was an increasing number of taxa in the soil and litter in Tarantulas as succession proceeds, successional stage had no effect on the richness of taxa found in the experimental decomposition boxes. The richness, equitability and diversity of the community were always higher in those boxes containing *P. americana* leaves regardless of the stage of succession. Because soils become more nutrient deficient with succession, late-successional species are more likely to invest in secondary compounds for protection of their leaves. These carbon-rich compounds lower the rate of leaf decomposition (Cornelissen *et al.*, 1999). Even though *P. americana* did not have a higher initial concentration of carbon or RF, it did accumulate recalcitrant materials at a greater rate than *P. chiapensis* during decomposition and decomposed at a lower rate. Because a correlation between low palatability for herbivores and low decomposition rate has been found in several studies (Grime *et al.*, 1996; Wardle *et al.*, 1998), one might expect that those substances that deter herbivores above-ground would also deter the macroinvertebrate fauna in the soil. The result that richness, equitability and diversity were always higher in those boxes containing *P. americana* leaves does not support this prediction. It is possible that most of the taxa that colonised decomposition boxes do not feed directly on the litter but constitute micro-organism grazers and predators. Despite the accumulation of recalcitrant materials in *P. americana,* more P and N were mineralised in this species, which may have been an indication of a higher microbial activity (Wardle, 1992) that in its turn may support a richer and more diverse macroinvertebrate community.

In lowland tropical rain forest in Mexico Barajas-Guzmán & Alvarez-Sánchez (2003) also found that, regardless of the age of the forest, there were consistent differences in the invertebrate communities invading decomposition bags with different foliar species. They did not find any difference in the decomposition rate of experimental litter between primary and secondary forest, but found that a higher abundance of invertebrates invaded decomposition bags containing *Nectandra abigens*, the species that lost the least mass during the experiment. However, their results differ from the ones presented here in that they did not find any significant difference in the diversity or richness of invertebrates that invaded decomposition bags containing different species.

It is possible that structural, rather than nutritional, properties of the *P. americana* leaves were favourable for the macroinvertebrate community. Anderson (1975) suggested that this may be the explanation why chestnut leaves and beech leaves had very different decomposition rates and yet sustained similar soil animal populations, since they provided similar structural habitats. Because pine needles and avocado leaves are structurally very different, it may be hypothesised that despite the slower decomposition rate, avocado leaves may sustain a richer tridimensional habitat for animals than needles that may form a denser uniform mass earlier in decomposition process. Results from the CCA indicated that this is unlikely because when the variance in community composition was partitioned among explanatory variables, litter chemistry was the set of variables that accounted for more variation (*c.*15%) while foliar species accounted for the least (*c.*3%).

As the experiment proceeded, the number of taxa increased in all decomposition boxes, while the diversity and equitability increased with time in the boxes placed in the 15- and 100-year-old forests only. Because these patterns were independent of the foliar species being decomposed, they are likely to be a result of differences in the seasonal variation of macroinvertebrate communities between successional stages. The only information available about seasonal variation in macroinvertebrate communities in Tarantulas is presented in the following chapter. Chapter 6 includes the results of monitoring the macroinvertebrate community through time in monoliths extracted from the 100-year-old forest exactly at the same dates when experimental boxes were collected for the experiment in this chapter (see figure 6.2). The seasonal variation will be further discussed in Chapter 6. For the present discussion it is sufficient to note that in the 100-year-old forest the soil macroinvertebrate taxa, equitability and diversity increased with time. This pattern coincides with that observed in the decomposition boxes retrieved from the 100-year-old forest. For the 15- to 75-year-old forests, it can only be speculated that the seasonal variation of the macroinvertebrate community may be different in mid-succession, probably as a result of the dominance of pine and oak litter.

The sets of variables explored in this experiment as explanatory of the community composition (successional stage, litter chemistry, collection date and foliar species) were similar to those explored in the chronosequence survey presented in Chapter 3. For the Tarantulas survey, of the total community variance, only 11.4% for the litter and 13.4% for the soil were explained. In contrast, 21.5% of the total variance in the community in experimental boxes was accounted for here. This is an indication that using litter that was monospecific and from only one decomposition cohort substantially diminished the amount of unexplained variance. In the natural environment, there must be a complex interaction between the diversity of litter and the variety of cohorts of decomposing organic matter, which has an important role in determining macroinvertebrate community composition.

In this experiment, the chemistry of the leaves accounted for more variance (*c.*15%) than any other set of variables. In fact it explained double the variance of the next highest set, the successional stage (7.5%). This result, together with the consistently higher diversity of macroinvertebrate taxa that invaded *P. americana* boxes, points to the nutrient release from decomposing litter as a major explanatory factor for the macroinvertebrate diversity and community composition in these forests. Because in this experiment the leaves from *P. americana* probably had substantially more nutrients than expected from naturally abscised leaves, conclusions about macroinvertebrate diversity in late succession can only be tentative, and will be revisited in Chapter 6 where the decomposition of abscised leaves in the 100-year-old forest is discussed. However, it is possible to conclude that the lower diversity of macroinvertebrate taxa in early successional stages where pine litter dominates is associated with the immobilisation of nutrients (and probable low microbial activity) occurring in this litter, particularly later than 15 years after disturbance.

Because it was found that *P. americana,* which mineralised more nutrients, had a more diverse macroinvertebrate community, it can be hypothesised that somehow later in succession richer resources are sustaining a rich macroinvertebrate community. At this stage, the availability of nutrients in decomposing litter may not be on average greater than in earlier stages because the abscised litter quality of the two late-successional species that I examined in Chapter 3 (*B. ovalis* and *Oreopanax xalapensis*) was relatively low. Although the rate of above-ground biomass increase has probably slowed down in late succession, the soils are already poor in readily available nutrients and therefore standing crop and litter quality are expectedly low. However, a substantial amount of semi-decomposed organic matter has accumulated in the soil through succession and the richness of the macroinvertebrate communities may be associated with the range of resource qualities available within this organic matter. Chapter 6 explores this idea in greater depth.

Although substantial differences were found in terms of number of taxa, equitability and diversity between collection dates, the composition of the community in decomposition boxes was not strongly driven by this factor, particularly in the 45 and 75-year-old forests. In the ordination spaces of individual successional stages, the community composition of different collection dates was more distinguishable in the 100- and 15-year-old forests than it was in mid-succession. This agrees with the suggestion above that in mid-succession there is less seasonal variation in the community composition than in than in early and late- succession, and this may be associated with the dominance of late-successional species in the litter. Differences in the community composition between successional stages were only significantly accounted by explanatory variables in the second half of the experiment and the boxes placed in the 15-year-old forest had the most distinct community, probably associated with the higher release of nutrients from the litter decomposed in this forest. All other successional stages had similar community compositions.

From these results it is not possible to support the hypothesis that the observed resemblance of the macroinvertebrate community in early-intermediate successional stages discussed in Chapter 3 is associated with the dominance of the litter from pine litter in those stages. It is only possible to conclude the most probable mechanism of influence by different foliar species on the community composition is through differences in the evolution of litter chemistry through decomposition. Independently of the litter being decomposed, the community composition in intermediate successional stages does not change significantly through time. This pattern may be related to a lower seasonal fluctuation of decomposer communities in those successional stages.

In litter where recalcitrant material is abundant (as in mature TMCF), specialised decomposers and comminuters may speed decomposition and nutrient mineralisation (Lavelle *et al.*, 1993). Substantial literature exists that explores thoroughly this issue through macroinvertebrate exclusion from decomposition litterbags and microcosm experiments (see for example Herlitzius, 1983; Coûteaux *et al.*, 1991 and Heneghan *et al.*, 1998; for review see Verhoef & Brussaard, 1990 and Berg *et al.*, 2001 for a modelling approach). All of these studies have concluded that the macroinvertebrate contribution to organic matter loss is mainly indirect through trophic interactions and that its importance varies according to climatic and litter quality constraints. Although the present experiment was not designed to test the effect of macroinvertebrates on the evolution of decomposition, it is feasible the richer and more diverse macroinvertebrate community in late succession may be counteracting the decline in decomposition rate expected from an increasing concentration of recalcitrant materials during the decomposition of late successional species such as *P. americana*.

In a microcosm experiment De Deyn *et al.* (2003) showed that macroinvertebrate communities promoted early grass successional communities to develop into more diverse late-successional communities by selectively feeding on the roots of dominant and highly competitive early-successional grass species and therefore favouring suppressed late-successional species. Although the experiment presented here does not address the question of whether macroinvertebrate communities have an effect on the course of succession in cloud forest, the fact that late successional stages sustain a higher number of taxa, together with the fact that the leaves of a late successional species attracted a more diverse macroinvertebrate community independently of where it decomposed, is in agreement with the suggestion that the macroinvertebrate community could preferably promote the release of nutrient from late successional litter, which in turn may benefit the cycling of nutrients locally under the canopies of these trees. This hypothesis is explored with the experiment presented in Chapter 6.

In the first year of decomposition tannin-protein complexes often accumulate in decomposing residue and their decomposition is extremely slow unless organisms capable of degrading them are present (Lavelle *et al.*, 1993). In nutrient poor forests (such as mature TMCF), leaves have high concentrations of tanins as lignin (Aerts, 1995). The fact that earthworms were common in pristine sites and absent from secondary forests (see Chapter 3) could have important repercussions in the decomposition of the litter, as has been observed in cleared forests in the humid lowland tropics (Tian, 1998). Earthworms have been characterised as ecosystem engineers because they can be highly influential in the physical properties of the soil including structural heterogeneity, stability, distribution of organic matter and hydrological characteristics (Lawton, 1994; Lavelle *et al.*, 1997). They are known to both have an important effect on N and C mineralisation, and their survival is affected by the nutrient quality of soil (Saetre, 1998). The fact that earthworms were almost completely absent during secondary succession for at least 100 years in El Rincón TMCF might be of paramount consequences for the cycling of nutrients and ecosystem services in disturbed forests. Because the study of decomposition presented here was restricted to the secondary succession between 15 and 100 years after disturbance, the importance of earthworms in the cycling of nutrients in undisturbed TMCF can only be inferred and should be given more attention in future studies.

Conclusion

The evidence shown here does not support the hypothesis that decomposition rate slows down as succession proceeds between 15 and 100 years after logging. However, pine litter dominating early-mid successional stages has a higher decomposition rate than the leaves from late successional plants and the higher availability of nutrients in early succession has a positive effect on the release of P, N and Ca from decomposing litter. This positive feedback between decomposition rate and nutrient availability is not sustained through succession. Nutrient sequestration evolves probably because the fast growing vegetation exhausts the reservoir of soil nutrients that became available as a result of the opening of the canopy and semi-decomposed organic matter accumulates down the soil profile.

Although leaves of a late-successional species (*Persea americana*) decomposed at a slightly slower rate than an early-successional species (*Pinus chiapensis*) in all successional stages, the number and Shannon's diversity of macroinvertebrate taxa that invaded decomposing *P. americana* leaves was consistently higher. The lower diversity of macroinvertebrate taxa in early successional stages where pine litter dominates maybe associated with the immobilisation of nutrients (and probable low microbial activity) occurring in this litter, particularly later than 15 years after disturbance.

No support was found for the hypothesis that the resemblance of the macroinvertebrate community in early-intermediate successional stages is associated with the dominance of pine litter. Independently of the litter being decomposed, the macroinvertebrate community composition in intermediate successional stages does not change significantly through time. This pattern may be related to a lesser seasonal fluctuation of the resident decomposer communities in those successional stages.