





## 1 **1. Introduction**

2 Most of the ash generated at present in Spain is produced in heat and power plants that  
3 adjoin pulp mills and particle and fiber board plants. Disposal of the ash in landfills is still the  
4 main method of managing the residue (Ministerio de Medio Ambiente, 2002). Although an  
5 heterogeneous material, wood and bark ash is in general highly alkaline and contains a wide  
6 variety of plant nutrients: calcium, potassium, magnesium and to a lesser extent phosphorus  
7 (Etiégni and Campbell, 1991), and generally low amounts of nitrogen and trace elements  
8 (Khanna et al., 1994; Vance, 1996).

9 In response to lower population densities in rural areas, as well as environmental and  
10 economical strategies, arable land is increasingly used for newly planted forests in the Iberian  
11 Peninsula. Chestnut is readily accepted for planting by private landowners, and the current  
12 plantation rate in Portugal, northern Spain and France has been estimated at 4500 ha per year.  
13 Chestnut is attractive to landowners because of the prospect of valuable timber production in  
14 relatively short rotations. The use of the ink-disease resistant hybrids, obtained by crossing  
15 *Castanea sativa*, *Castanea crenata* and *Castanea mollissima* became popular around 1960,  
16 and several clones have been selected for their desirable characteristics in terms of timber  
17 production, i.e., straightness, branchiness and rapid height growth. Nutritional problems in  
18 southern European forest plantations (Zás and Serrada, 2003) and the capacity of ash to  
19 improve growth on N-rich mineral soils and peat soils (Ferm et al., 1992, Jacobson, 2003) led  
20 to particular interest in the use of wood ash in broadleaf plantations.

21 Ingerslev et al (2001) considered that there was an urgent need for research concerning  
22 recycling of the nutrients by the application of wood ash, in light of the widespread use of  
23 forest residues for energy production. There is much information about the effect on tree  
24 growth of wood ash applied to peat soil (Moilanen et al, 2002, 2004) and mineral podzolic  
25 soils (Jacobson, 2003; Saarsalmi et al, 2006), but there is very little information about the

1 growth of temperate broadleaf species following the application of wood-bark to mineral  
2 soils. Furthermore, there is still a need to analyse the long term effects of ash application, and  
3 to consider the spatial heterogeneity usually present in forest trials.

4 The aim of the present study was to assess the effect of two doses of ash on the growth  
5 of a hybrid chestnut plantation in two consecutive periods of 3 and 4 years, while considering  
6 the spatial autocorrelation of the data, and the relationship between growth and the foliar and  
7 soil nutrients.

## 8 **2. Materials and methods**

### 9 **2.1. Site description**

10 The study was carried out in a hybrid chestnut plantation aged 5 years in July, 2001.  
11 The plantation was established in a former grassland, in which the soil was prepared by  
12 rotovating and subsoiling, then planting of layers 1.2 to 1.5 m height, at a regular spacing of  
13 5x5 m, to provide a density of 400 trees ha<sup>-1</sup>. Fertilization was carried out at establishment, by  
14 placing 50 g of a controlled release fertilizer (9-13-18-2: N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O and MgO) in the hole  
15 at planting. Weed control (by chopping rollers) and low pruning to a height of 1.5 m were  
16 carried out as tending operations, always with the main goal of timber rather than nut  
17 production.

18 The plantation is located near the city of Santiago de Compostela, northern Spain (42°  
19 54'N, 8° 16'W, 330 m asl.). The average annual precipitation in the area is 1,836 mm and  
20 mean annual temperature is 12.8 °C. The soil is developed on biotitic schists and, according to  
21 the FAO-UNESCO system is classified as an Umbrisol, with an A<sub>h</sub> horizon at a depth of 0–25  
22 cm and an AB horizon of 25–60 cm depth. The surface horizon of the soil is characterized by  
23 being acidic (pH 5.2) with a high content of organic matter (13.1%), loam texture (7.2% of  
24 coarse sand, 37.8 % of fine sand, 42.1% of loam, 12.8% of clay), and slightly low  
25 concentrations of available cations. The C:N relation was 8.6, well below the usual values for

1 granitic rock in the area. The understory consisted of a complete cover of herbaceous  
2 vegetation, mainly *Dactylis glomerata*, *Plantago lanceolata* and *Holcus mollis*.

### 3 **2.2. Experimental design**

4 The ash was obtained from a paper pulp processing plant and was mainly derived from  
5 the combustion of bark (70% *Pinus* spp. and 30% *Eucalyptus* spp.). The composition of ash  
6 and the corresponding amounts of elements applied to the soil are shown in Table 1. The main  
7 properties of this product are as previously described (Solla-Gullón et al, 2006). The ash was  
8 applied in July 2001, when the plantation was 5 years old, before canopy closure, by use of a  
9 small rotary spreader with a 400 kg graduated hopper.

10 The study was carried out as a factorial design with four blocks and three treatments.  
11 The size of each plot was 35 x 35 m and the treatments included no fertilization (control) and  
12 two doses of MWBA, D10 (10 Mg dry weight ha<sup>-1</sup>) and D20 (20 Mg ha<sup>-1</sup>). In relation to the  
13 total nutrient requirements for forest production calculated for a chronosequence of chestnut  
14 coppice (Ranger and Colin-Belgrand, 1996), D10 would add only a quarter of the P and K  
15 requirements, two-thirds of Mg and all the Ca requirements, whereas D20 would add half of  
16 the P and K requirements and all the requirements in Ca and Mg (see Table 1). The  
17 concentration of P was low, as usually reported for wood and bark ash (Demeyer et al, 2001).  
18 The low amount of N in the MWBA was acceptable because of the high availability of N in  
19 the soils under study.

### 20 **2.3. Sampling and analysis of plants and soil**

21 Soil samples were collected just before and one month after ash application, then  
22 every three months for 27 months (June 2001 to September 2003). No further samples were  
23 taken because of rapid convergence of the treatments. Samples from the upper mineral  
24 horizon (20 cm) were collected at random from six points and were mixed to obtain one  
25 composite sample per plot. Soil samples for chemical analysis were air dried and sieved with

1 a 2 mm screen. Total C, N and S were analysed with a LECO CNS Analyzer. Available P, Ca,  
2 Mg, K, Mn, Fe, Cu, Ni and Zn were extracted by the Mehlich 3 procedure (Mehlich, 1984)  
3 and were determined by ICP-OES.

4 Leaf samples were collected twelve times, starting in July 2001, i.e., five growing  
5 seasons after plantation. The samples were collected three times a year (April, July and  
6 September) in 2001, 2002 and 2003. Undamaged and full-sized leaves were sampled from  
7 dominant and codominant trees in the upper third of unshaded crowns in the plot. Each  
8 sample consisted of 100 leaves, which were pooled to provide a composite foliage sample for  
9 each plot, so that there were 12 samples per date. Foliar samples were oven-dried (65°C) to a  
10 constant weight, milled (0.25 mm) and extracted with H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> (Jones et al., 1991). The  
11 concentrations of P, K, Ca, Mg, Mn, Fe, Cu, Ni and Zn in the leaf extracts were determined  
12 by ICP-OES. The contents of N and S in solid milled material were determined in a LECO  
13 Analyzer.

14 To determine the nutritional status of the trees, the reference foliar levels for *Castanea*  
15 *sativa* proposed by González-Rodríguez (1975), Leonardi et al (1996) and Portela et al (2003)  
16 were used. Comparisons were also made with the mean levels observed for young plantations  
17 of this species in Galicia by Álvarez et al. (2004). References values are shown in Table 2.  
18 The second approach used for foliar analysis was the vector analysis technique (Timmer and  
19 Stone, 1978), in which the relative concentrations and relative nutrient contents in biomass  
20 are presented graphically, considering the control as the comparison level. This method  
21 allows the identification of dilution (slight increase in content with decreasing concentration),  
22 sufficient levels (increase in content without change in concentration), deficiency (increase in  
23 content with slight increase in concentration), luxury consumption (increase in content and  
24 concentration at the same rate) and even excessive levels or toxicity (in the case of decreasing

1 content). The dry weight of each leaf was estimated from the weight of 100 representative  
2 leaves.

### 3 **2.4. Tree measurements and data analysis**

4 The breast height diameter and total height were measured in April 2001, September  
5 2003 and again in July 2008 in all trees within the plots. The diameter at breast height was  
6 measured in two directions on all the trees, to within 1 mm. Tree height was measured with  
7 telescopic measuring rods, to within 10 cm. Cross measurement of crown diameter was  
8 determined in July, 2008, and the geometric mean calculated (DC12). Increments were  
9 calculated as differences between measurements; the variables considered for analysis were  
10 the diameter and height increment between age 5 and 8 years (ID8 and IH8) and the  
11 increments between ages 8 and 12 years (ID12 and IH12). As the trees were planted following  
12 a regular 5x5 m grid, the approximate x–y coordinates of each tree were recorded considering  
13 their position in the block.

14 Residuals of each variable after subtraction of the treatment effects were used to  
15 explore the spatial structure of the data. A one-way analysis of covariance with the treatment  
16 effect considered as fixed was carried out with the initial diameter (or height) as the covariate,  
17 by use of the GLM procedure of SAS (SAS Institute 2004). The spatial structure of the  
18 resulting residuals was modelled with a semivariogram, which plots the semi-variance among  
19 trees as a function of the distance between them (Cressie 1993). The semi-variance  $\gamma(h)$  was  
20 calculated as:

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^n [z(s_i) - z(s_{i+h})]^2 \quad (1)$$

21 where  $n$  is the number of pairs of neighbouring trees separated by distance  $h$  (called the lag  
22 distance),  $z(s_i)$  is the measured variable for a tree located at  $s_i$ , and  $z(s_{i+h})$  is the value for a tree  
23 located at a distance  $h$  from  $s_i$ . Experimental semivariograms were constructed by use of the  
24 VARIOGRAM procedure in SAS (Sas Institute, 2004). An exponential theoretical

1 semivariogram  $\gamma(h)=c_n+c_0(1-e^{-h/a_0})$  was fitted to the experimental semivariogram by the NLIN  
2 procedure in SAS (SAS Institute 2004).

3 The variation of residuals was parted into spatially autocorrelated variation and  
4 random error by kriging. The kriging values at each tree location were used to correct the  
5 original values of each variable in relation to their spatial variation. The kriging analysis was  
6 performed by the KRIG2D SAS procedure (SAS Institute 2004).

7 Values adjusted for the spatial structure were then analysed with the following random  
8 model:

$$y_{ijk} - k_{ijk} = x_{ijk} + \mu + T_i + B_j + TB_{ij} + \delta_{ijk} \quad (2)$$

9 where  $y_{ijk}$  is the value of the original variable of the  $k$ th tree in the  $j$ th block and the  $i$ th  
10 treatment,  $k_{ijk}$  is the kriging estimate at the position of that tree,  $x_{ijk}$  is the covariate,  $\mu$  is the  
11 overall mean,  $F_i$ ,  $B_j$ , and  $FB_{ij}$  are the random effects of treatment  $i$ , block  $j$ , and the  
12 interaction, and  $\delta_{ijk}$  is a spatially independent error. The same statistical model was also used  
13 to analyse uncorrected original values. The GLM procedure of SAS (SAS Institute, 2004) was  
14 used for analysis of covariance. The use of Eq 2 results in diameter increments adjusted for  
15 differences in initial diameters and therefore, treatments were tested for their impacts on  
16 adjusted means. Tukey's studentized range (HSD) was employed.

### 17 3. Results

#### 18 3.1. Effect of ash in tree growth and mortality

19 The residuals obtained after subtracting treatment effects revealed non-random spatial  
20 structures. The semivariograms revealed spatial autocorrelation, but of pattern and intensity  
21 that varied greatly among the different growth variables and periods, as is shown in Fig. 1.  
22 Spatial heterogeneity was much more evident for the first growth period, as revealed by  
23 higher path-nugget variance ratios ( $c_0/c_n$ ), but was also present for the second period. Height  
24 growth had a greater intensity of spatial structure than diameter growth.

1 The effective range ( $3a_0$ ) or patch size of the exponential theoretical semivariograms  
2 was also variable, although generally lower than the block side (35 m) for the first growth  
3 period, indicating spatial heterogeneity within blocks that implies a violation of the block  
4 design assumptions. As an example, the spatial variation in ID8, modelled by kriging, is  
5 shown in Fig. 2.

6 The results of the ANCOVA are shown in Table 3, for both the non-spatial and the  
7 two spatial analyses. The initial height or diameter value was always considered as a covariate  
8 in the analysis to explain growth. The interaction was completely removed when the position  
9 of each tree was considered in the analysis using the iterative kriging. The effect of the  
10 treatment was much clearer after spatial analysis, and the least-squares means (LSmeans)  
11 classification provided a better understanding of the differences between treatments.  
12 Application of the ash enhanced the diameter and height growth in the first 3-year period,  
13 with better growth as the dose increase. For the second 4-year period, the effect was still  
14 significant, but only the D20 dose provided a response that was significantly different from  
15 the control. The block effect, which was always significant for the non-spatial analysis, was  
16 removed by kriging in the case of ID8 and IH8.

17 Seedling mortality after ash application was analysed throughout the study. Average  
18 mortality was 4.1% (D20), 7.1% (D10) and 17.7% in the control plots. The combined effect  
19 of mortality and crown width led to a clear increase in the percent of canopy cover (58.1% in  
20 D20, 64.7% in D10 but only 35.7% in the control plots).

### 21 **3.2. Growth in relation to nutrition**

22 Foliar N and P concentrations in the control plots were between 18-21 and 1.7-3.5 mg  
23  $g^{-1}$ , respectively, which can be considered as sufficient for this species (Table 2). The  
24 application of ash had no significant effect and the vector analysis indicated that both  
25 elements were sufficient for satisfactory growth of the plantation (data not shown). Foliar Ca

1 concentrations were low in the control plots, between 5.5 and 8 mg g<sup>-1</sup>, and ash application  
2 did not have any effect on the concentrations. The concentrations of K ranged from 4.3 to 7  
3 mg g<sup>-1</sup>, clearly below the marginal values proposed for the species, also with no increase in  
4 concentrations resulting from ash application. In the case of Mg, the range was between 2.2  
5 and 3.6 mg g<sup>-1</sup>, well above the marginal concentration of 2 mg g<sup>-1</sup> proposed for chestnut.  
6 Again ash application did not have any significant effect. For the last three elements, the  
7 vector analysis provided a positive response in terms of relative content and also relative  
8 concentration, indicating a slight positive effect in terms of nutrition (Figure 3).

9 Ash application had a significant effect on soil pH, which increased by up to 0.6 units  
10 (for the highest dose) in relation to the control plots (Figure 4). The effect was very short-  
11 lived, and the pH returned to values close to the initial values after only 18 months. The ash  
12 fertilization had a short term effect on the available concentrations of Ca, Mg and K, and was  
13 only significant in the case of K (Figure 4). The initial level of Ca was 535 mg kg<sup>-1</sup>, a high  
14 level for the region, attributed to former use of the land as grassland. This was almost doubled  
15 by both doses of the ash, and the effect of the highest dose lasted longer. By July 2003, i.e.,  
16 24 months after fertilization, the levels returned again to the initial values. The initial  
17 concentration of available Mg was 54 mg kg<sup>-1</sup>, also quite high for the region and it was also  
18 almost doubled by the fertilization, again with return to initial values after 24 months. The  
19 initial concentration of K was 75 mg kg<sup>-1</sup>, close to the average levels for soils in the region,  
20 and there were no signs of previous fertilization with this element. The ash application led to  
21 very important increases in this element, exceeding 200 mg kg<sup>-1</sup> for the dose of 10 t ha<sup>-1</sup> in  
22 many samples. The effect was also short-lived and had almost disappeared after 24 months.

#### 23 **4. Discussion**

24 Growth variables are very often affected by spatial heterogeneity. In this study, the  
25 intensity of the spatial structure was higher for the first period than for the second period; this

1 appears reasonable if we consider that growth in the last phase is in fact much more affected  
2 by individual competition, as the plantation reaches canopy closure. Height growth displayed  
3 greater spatial correlation than diameter, as also found in genetic trials (Zas, 2006). The block  
4 structure of the randomized complete block design was not sufficient to account for this  
5 spatial heterogeneity, because the pattern of spatial structure was in small patches in relation  
6 to longer blocks, which were clearly too heterogeneous. The kriging procedure was able to  
7 remove the spatial variation.

8         The results of the present study showed that the ash treatment significantly affected  
9 growth of this young chestnut plantation. Although the duration of the effect is questionable,  
10 the increase in early growth can bring forward canopy closure in the plantation, thereby  
11 helping to check the growth of competing vegetation and reduce the risk of fire. Improved  
12 growth of tree species in response to wood-bark ash application has also been reported in  
13 studies of other forests growing in colder climates (Ferm et al., 1992; Moilanen et al., 2002)  
14 and, particularly, in fast growing species plantations when the ash is applied before canopy  
15 closure (Solla-Gullón et al, 2006). Taking into account the results obtained in the present  
16 study and from ash application in other fast growing plantations (Solla-Gullón et al., 2008),  
17 and considering the efficiency of the addition of ash, application of 10 Mg ha<sup>-1</sup> of ash appears  
18 sufficient for each operation. In this study, the higher dose still had a clear effect after 7 years,  
19 although the repeated application of wood-bark ash in moderate amounts throughout the  
20 rotation appears more appropriate. The dose of 10 Mg ha<sup>-1</sup> is higher than those reported in  
21 other colder climates and with less intensive silvicultural systems, in which doses of 3–7 Mg  
22 ha<sup>-1</sup> are usually recommended (Hytönen, 2003).

23         The chemical soil properties of the chestnut habitat in Spain have been defined by  
24 Gandullo et al. (2004), but plantations in the northwestern area usually have values higher  
25 than the upper thresholds for the organic matter and soil total nitrogen (Blanco et al, 2000). It

1 should be also considered that the plots had not yet reached canopy closure, and thus nutrient  
2 requirements of the stand were high in comparison with biomass production. After canopy  
3 closure, highly efficient nutrient recycling under low availability conditions can be expected  
4 (Ranger and Colin-Belgrand, 1996). Although European chestnut is known to grow on  
5 naturally acid soils, nutrient deficiencies can occur as a consequence of poor nutrient reserves  
6 or nutrient imbalances, leading to detrimental effects on tree growth and nut production  
7 (Portela et al. 2003). The results observed in the present study revealed a positive effect of ash  
8 fertilization in terms of Ca, Mg and K nutrition, even though the plantation was established on  
9 former agricultural land, in which there are generally no strong soil limitations for the species.  
10 The results are consistent with those obtained by Laroche et al (1997), who showed that  
11 addition of Ca and Mg had a positive effect on seedling growth.

## 12 **5. Conclusions**

13 The results of this 7-year-long study showed that the application of wood-bark ash  
14 improved the growth and nutritional status of a young hybrid chestnut stand. A dose of 10 Mg  
15 ha<sup>-1</sup> repeated twice along the rotation appears appropriate for acid mineral soils in abandoned  
16 agricultural lands.

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1 **Tables**

2 Table 1. Chemical composition of the ash used in the study.

	Total <sup>(a)</sup>	Available <sup>(b)</sup>	Applied in wood ash 10/20 Mg ha <sup>-1</sup> (kg)
Organic matter <sup>(c)</sup> (%)	42.4		
C (g kg <sup>-1</sup> )	116.3		1163 - 2326
N (g kg <sup>-1</sup> )	1.9		19 - 38
S (g kg <sup>-1</sup> )	0.3		30 - 60
P (g kg <sup>-1</sup> )	5.2	0.07	52 - 104
Ca (g kg <sup>-1</sup> )	99.7	32.9	997- 1994
Mg (g kg <sup>-1</sup> )	20.2	5.0	202 - 404
Na (g kg <sup>-1</sup> )	7.7	6.5	77 - 154
K (g kg <sup>-1</sup> )	30.2	27.9	302 - 604
Al (g kg <sup>-1</sup> )	15.1	n.d	151 - 302
Cd (mg kg <sup>-1</sup> )	3.6	0.9	0.04 – 0.08
Cr (mg kg <sup>-1</sup> )	20.7	3.1	0.21 – 0.42
Cu (mg kg <sup>-1</sup> )	54.7	15.1	0.55 – 1.1
Fe (mg kg <sup>-1</sup> )	1385	26.7	13.8 – 27.7
Mn (mg kg <sup>-1</sup> )	6173	106.3	61.7 – 123.4
Ni (mg kg <sup>-1</sup> )	118.6	43.0	1.2 – 2.4
Pb (mg kg <sup>-1</sup> )	79.0	22.0	0.79 – 1.6
Zn (mg kg <sup>-1</sup> )	334.7	35.2	3.3 -6.6
Hg (mg kg <sup>-1</sup> )	< 1		< 0.01

3 Moisture content (%): 11.2; pH (H<sub>2</sub>O): 13.

4 <sup>(a)</sup>Digestion in HNO<sub>3</sub>; <sup>(b)</sup> Extraction with Mehlich 3; <sup>(c)</sup> Calcination

5

1 Table 2. Reference range and foliar concentrations in chestnut (*Castanea* spp.) plantations in  
 2 Galicia (NW Spain) for *Castanea* ssp plantations.

	Reference <sup>a,b,c</sup>	Young plantations (NW Spain) <sup>d</sup>
N (mg g <sup>-1</sup> )	15 - 25	23.6
P (mg g <sup>-1</sup> )	1.3 - 1.8	1.6
K (mg g <sup>-1</sup> )	8 - 13	7.3
Ca (mg g <sup>-1</sup> )	5 - 8	5.1
Mg (mg g <sup>-1</sup> )	1 - 2	2.4
Fe (mg kg <sup>-1</sup> )		113
S (mg kg <sup>-1</sup> )		1.7
Mn (mg kg <sup>-1</sup> )		60
Cu (mg kg <sup>-1</sup> )		4
Zn (mg kg <sup>-1</sup> )		27

<sup>a</sup> González Rodríguez (1975), <sup>b</sup> Leonardo et al (1996), <sup>c</sup> Portela et al (1999), <sup>d</sup> Álvarez et al (2004).

1 Table 3. Results of the ANCOVA analysis before and after removal of the spatial effect.  
 2

Variable	p level of ANCOVA factors. No spatial structure considered				LS means classification		
	Covariate	Treatment	Block	Interaction	C	D10	D20
ID8	<0.0001	<0.0001	<0.0001	<0.0001	3.45a	3.79a	4.22ab
ID12	<0.0001	0.0473	<0.0001	0.0054	7.57	7.54	8.70
IH8	<0.0001	0.0009	0.0027	<0.0001	1.54a	1.71b	1.80b
IH12	<0.0001	0.0029	<0.0001	<0.0001	3.21a	3.63ab	3.98b

Variable	p level of ANCOVA factors. Iterative kriging method				LS means classification		
	Covariate	Treatment	Block	Interaction	C	D10	D20
ID8	<0.0001	<0.0001	0.674	0.673	3.44a	3.75b	4.25c
ID12	<0.0001	0.0219	0.0095	0.504	7.92a	7.61a	8.82b
IH8	<0.0001	<0.0001	0.0187	0.605	1.57a	1.69b	1.81c
IH12	<0.0001	0.0264	<0.0001	0.091	3.45a	3.68ab	3.97b

3

4

1     FIGURE CAPTIONS

2     Figure 1. Examples of the semivariograms of residuals after subtracting treatment effects in a  
3     first iteration for the four variables analysed in the study. The reduction in semivariance at  
4     short distances indicated a patchy structure. The effective range ( $3a_0$ ) and the nugget value  
5     ( $c_n$ ) are represented as vertical and horizontal lines, respectively. The intensity of the spatial  
6     structure ( $c_0/c_n$ ) is shown for each graph.

7  
8     Figure 2. Plot of height residuals after subtracting treatment effects in a fertilization trial of  
9     chestnut trees, showing nonrandom spatial variation and modeling of this variation through  
10    iterative kriging. White values represent dead trees. Five tones of grey are used to represent  
11    the total range of residuals and the residuals estimated by kriging, with black points  
12    representing the highest values of the original variable. Black lines are the block boundaries.

13  
14    Figure 3. Vector analysis diagrams for the effects of wood ash treatments on foliage Ca, Mg  
15    and K in the young chestnut plantation. Relative concentrations and contents are referred to  
16    100 for the control. The treatments are represented by ● for control, ▲ for D10 WBA, ◆ for  
17    D20 WBA. The arrow shows the main trend of change derived from ash application.

18  
19    Figure 4. pH and available concentrations of Ca, Mg and K in soils, for the different  
20    treatments throughout three growing periods. On the x-axis, J is July, S is September, D is  
21    December and M is March. The treatments are represented with ● for control, Δ for D10  
22    WBA and ▲ for D20 WBA. Different letters show significant differences between treatments  
23    at each sample date ( $p < 0.05$  in Tukey's test).

Figure

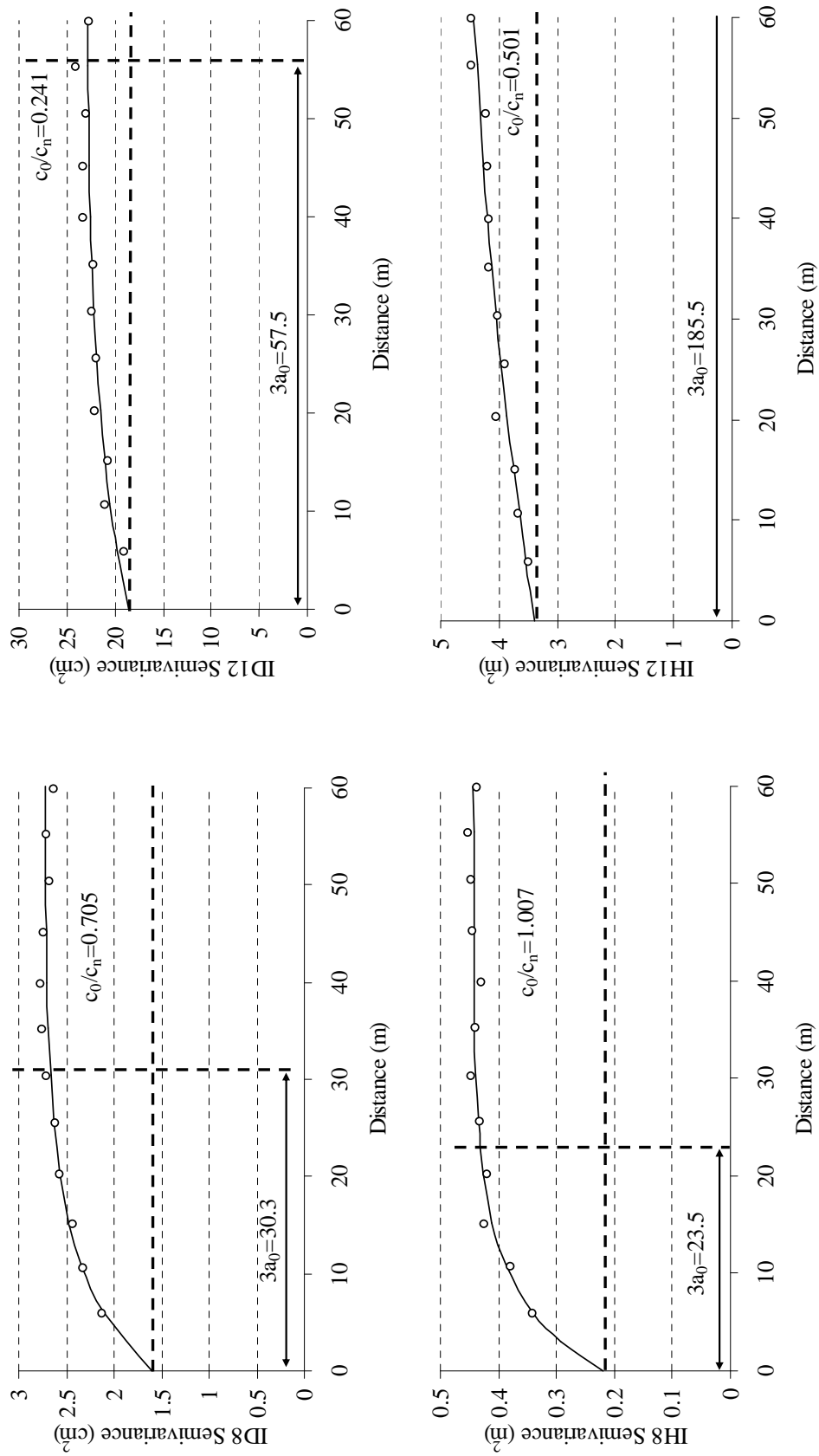


Figure 1

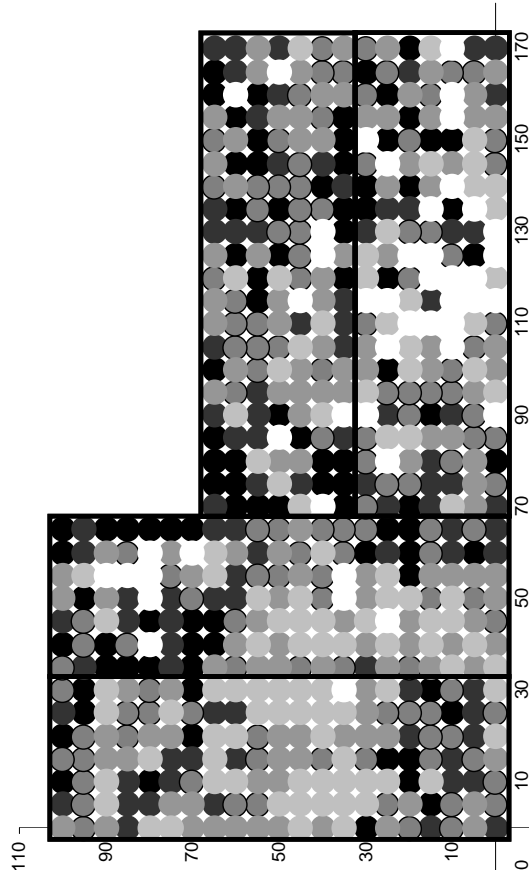
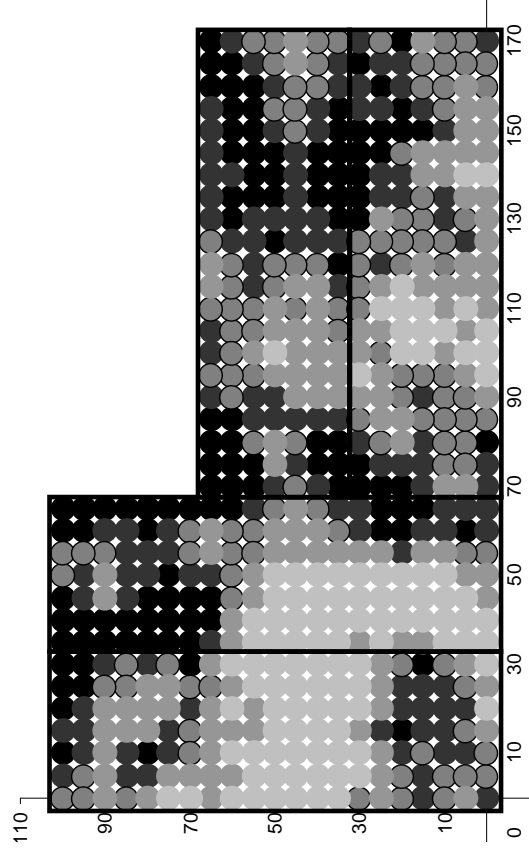


Figure 2

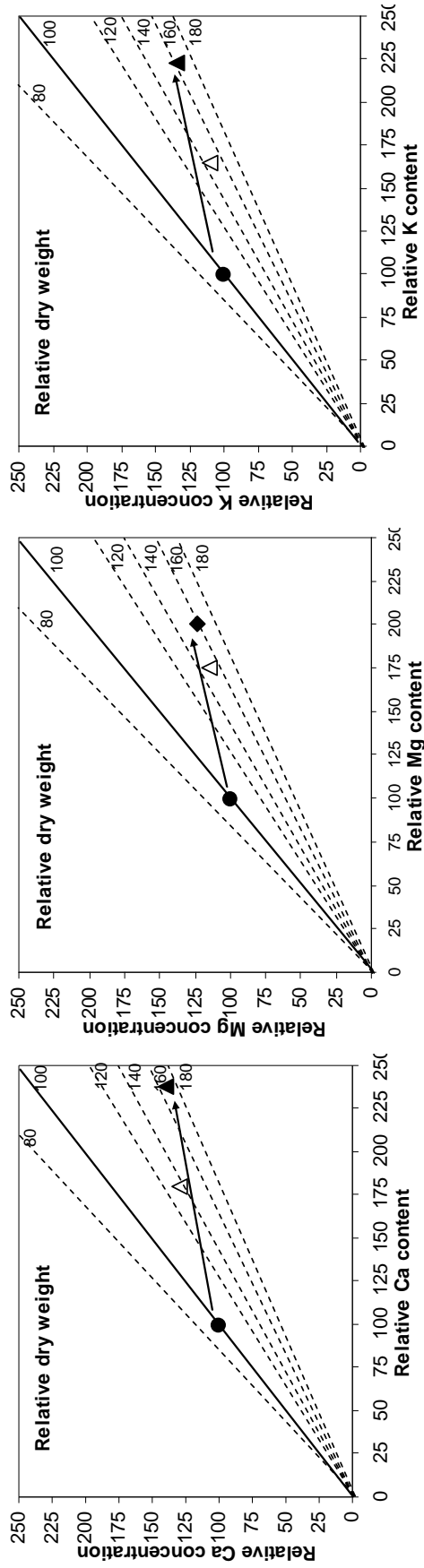


Figure 3

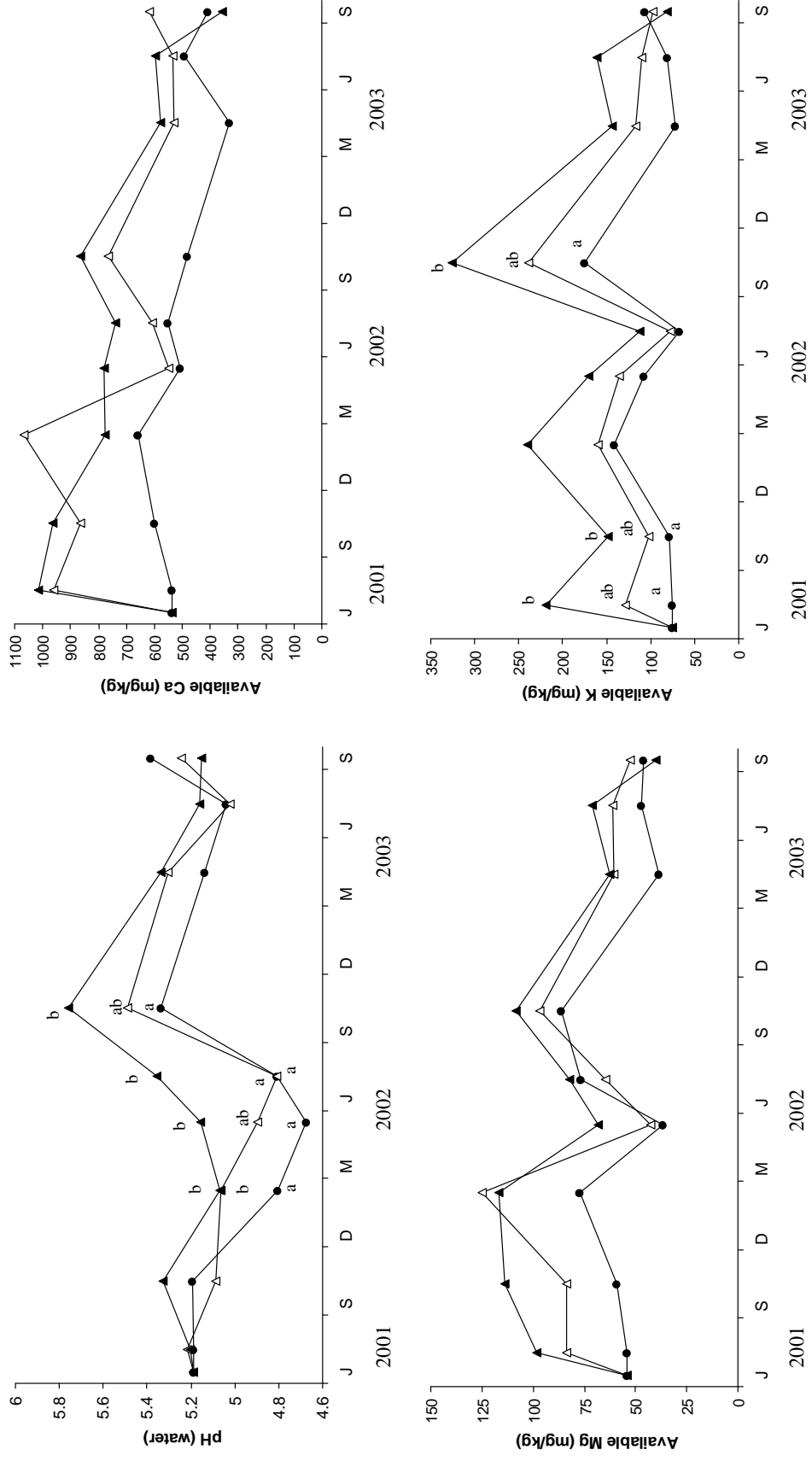


Figure 4