Combined application of calcium carbonate and NPKS fertilizer improves early-stage growth of poplar in acid soils

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Abstract: The cultivation of *Populus* spp. on acid soils is difficult mainly due to low nutrient availability, limiting the distribution and use of this marketable tree species. In this paper we report the results of two experiments, in which a granulated highly reactive micronized calcium carbonate ($CaCO_3$) was tested at increasing levels to improve the effect of NPKS fertilization on poplar growth. Twin field and pot experiments were carried out in 2017 using two different poplar clones, both of which are often used in Italy. In addition to analysing the data from the two experiments separately, common patterns were evaluated using a mixed-effect model with $CaCO_3$ level and fertilization as fixed effects, and the experiment type as random effect. Growth was assessed in terms of total height, diameter and biomass. Taken together, the results from the two experiments showed that fertilization led to enhanced growth of poplar, but this effect was stronger when soil conditions in terms of pH and exchangeable Ca were at a sufficiently high level. Available nutrient concentrations in the soil and foliar nutrient concentrations in the plant suggested co-limitation of poplar growth by N and Ca. In conclusion, the results of this study on one hand emphasize the importance of adapting the level of $CaCO_3$ to the given soil conditions, and on the other hand ask for further studies addressing the relative importance of elevated pH and improved Ca nutrition.

Keywords: poplar growth, calcium carbonate amendment, calcium nutrition; NPKS fertilization; plant nutrient status; soil pH; soil nutrient availability

1. Introduction

Fast-growing hybrid poplar plantations have become very important worldwide over the last decades due to an increasing demand for timber and the need to reduce related environmental impacts of forestry (Winans et al., 2015). In 2015, the total area of planted poplar accounted for 31.4 million ha all around the globe, of which 58% were managed for multi-purpose, 30% for industrial roundwood supply, 9% for environmental protection and 3% for energy wood (FAO, 2016). When established on nutrient-poor soils, poplar plantations require extensive nutrient management to reach their full productivity potential and, as a common practice, many commercial plantations are therefore fertilized at their establishment with a standard NPKS fertilization (Bergante et al., 2020; Hacke et al., 2010). Fertilization is usually performed within the first 4-5 years while fertilization prescriptions vary according to site fertility and other variables, e.g. water availability. Positive effects associated with fertilization on aboveground biomass yield in young poplar stands cultivated on arable land have been reported by Coleman et al. (2006) and Georgiadis et al. (2017), but there were also some studies with no or little effect of fertilization (Bergante et al., 2020; Jug et al., 1999).

Climate and soil characteristics are known to influence the plant availability of soil nutrients and thus the efficacy of fertilization (Doty et al., 2016; Rennenberg et al., 2010). Soil pH is a major factor known to influence nutrient availability (Böhlenius et al., 2020). In extremely acid or alkaline soils, nutrient availability is often low, and as a consequence, plant growth reduced (Hjelm and Rytter, 2016). At pH lower than 5- 5.5, the availability of many nutrients including the major nutrients N, P, K, Ca, and Mg is limited. In particular, the fixation of nutrients occurring in anionic form such as P at low pH and the respective limiting effect on fertilization have been demonstrated, e.g. by Fernández and Hoeft (2009). As a consequence, fertilization of acidic soils often fails to achieve its purpose and may in addition lead to environmental problems due to increased risk associated with the leaching of cationic nutrients (Beaudoin et al., 2005). In this framework, liming is a common agronomic practice performed on soils with low pH values to improve nutrient availability, as well as to address the issues of nutrient fixation and Al toxicity. For this purpose, different calcium (Ca) containing compounds can be used such as $CaCO_3$, $Ca(OH)_2$, $CaMg(CO_3)$, CaO and all have been shown to be able to raise the pH and to mobilize some nutrients (Holland et al., 2018). If applied correctly in terms of quantity and time, this soil amendment has multiple advantages, including mobilization of anionic nutrients and thus improving their plant uptake, decreasing leaching losses of cationic nutrients, and enhancing soil microbial activity. While some studies on growth response of fertilized poplar seedlings have proven that the uptake of added nutrients can be increased by raising soil pH (Foster and Bhatti, 2006; Han et al., 2016), other studies have not shown any effect of liming on growth of forest trees (Reid and Watmough, 2014). An important additional effect can be improved Ca nutrition of the plants, since exchangeable Ca is inherently low in acid soils (Foster and Bhatti, 2006; De Vries and Posch, 2011), and reductions in exchangeable Ca have been associated with growth declines in several woody species (Schmidt et al., 2015). Calcium is an important macronutrient required by trees, in particular by poplar. For this species, Lautner et al. (2007) demonstrated that Ca acts as an important regulator in many processes related to growth and wood formation and responses to environmental stresses. Nevertheless, the appropriate levels of Ca dosage in forest plantations and tree farming are still unknown for many tree species (Grover et al., 2021).

In this study, we address the question how different combinations of liming and fertilization affect the growth of hybrid poplar trees and how this is related to pH and the availability of Ca and other major nutrients. Considering the scientific evidence for the importance of calcium addition on wood formation discussed above and the contradictory results with respect to growth response following liming, we investigated the effect of calcium addition in form of calcium carbonate ($CaCO_3$) in combination with standard NPKS fertilization on growth and nutrient uptake of poplar in acid soils. Specifically, based on results from a field and pot experiment, we test the hypothesis that the growth of poplar on acid soils can only be increased by fertilization if soil pH is raised sufficiently by $CaCO_3$ to (i) minimize the abundance of anionic exchange sites thus increasing particularly the availability of P, and (ii) to increase the concentration of exchangeable Ca.

2. Materials and Methods

2.1 Experimental design

In early April of 2017, two twin experiments, both with a randomised split-plot design comprising 4 replicates, were realised in Northern Italy. Two poplar clones of recent wide commercial use in Italy were employed. Cuttings with an average diameter of approximately 1.5 cm and a length of about 22 cm were collected from a nursery in Casale Monferrato in late 2016. In the first experiment cuttings of the ‘Orion’ clone (*Populus ×canadensis*) were planted in open field at “Mezzomerico” (MER; 45°38'09.1"N 8°35'18.8"E). This trial covered a total area of 2,000 m^2 with 4 blocks of 27.5 m x 18 m (Fig. 1). Inside each block, 10 experimental units of 9 × 5.5 m were defined, one for each fertilization x calcium carbonate treatment (see below). For practical reasons, in each block the same sub-blocks of 5 units were fertilized or unfertilized, while the 5 calcium carbonate levels were assigned randomly within each sub-block (Fig. 1). In each experimental unit, 24 cuttings were planted in 3 rows. The spacing was 3 m between planting rows and 0.65 m between individual plants within a given row. This spatial design is the typical layout used in Italy for a Short Rotation Coppice plantation. An additional spacing of 1 m between the two plants in a given planting row belonging to different experimental units was added to ensure the absence of edge effects between treatments. A composite soil sample from the field was composed of 4 % coarse sand, 22% fine sand, 30% coarse silt, 26% fine silt and 10% clay (silty loam), and the pH (in H_2O) without amendment was 4.4.

The second experiment used cuttings of the 'AF6' clone (*Populus ×generosa* × *Populus nigra*) which were planted in 40 plastic pots with a diameter of 29 cm and a volume of 15 dm^3, kept at the experimental farm "Mezzi" (MEZ; 45°08'10''N, 8°30'44''E), using soil material collected from the upper 20 cm at a specific location at the site MER, where the field experiment was established. This soil had the same texture but a higher pH (4.7) than the composite sample of the field experiment (measured for an aliquot of the mixed soil used for filling the pots). Each of the 4 blocks consisted of 10 individual pots, one for each fertilization x calcium carbonate treatment (see below), grouped in fertilized and unfertilized sub-blocks and relative positions of calcium carbonate levels randomly assigned within each sub-block. In each pot, 4 cuttings were planted.

The fertilization consisted of nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) (NPK(S) 15.15.15.(5)), i.e. 15% N (4% NH_4, 11% Urea), 15% P_2O_5 (5% soluble in ammonium citrate and water, 10% soluble in mineral acids), 15% K_2O soluble in water and 5% of SO_3 soluble in water, and was applied at a rate of 100 kg ha^{-1} each of N, P_2O_5, and K_2O and 33 kg ha^{-1} of SO_3. This type of fertilization is the most common practice in Italy. The 5 calcium carbonate levels were 0, 500, 1000, 1500, and 2000 kg ha^{-1}, herein referred to as C0, C500, C1000, C1500 and C2000, respectively. The calcium source used in this study was Omya Calciprill®, a granulated highly reactive micronized calcium carbonate ($CaCO_3$). The granules had an average size of 2-6 mm and consisted of $\geq$ 91% chalk present in form of micronized particles with a weighted median particle size of d50 = 4.5 μm. Calcium carbonate amendment and fertilizer were spread manually; the former was distributed only during establishment of the trial, while fertilizer was spread at the end of May (late Spring) of the first and second year of growth for the field experiment. In the pot experiments, irrigation combined with insecticide treatment was applied twice per week. The pH of the irrigation water was 7.74, as measured at the beginning of the experiment. In the open field, weeds were controlled using herbicides after planting and rotovators during summer. The field experiment was not irrigated but received only rainfalls.

2.2 Data collection

At the end of the growing season, the diameter 20 cm above the collar (plants in pot trial), and diameter at breast height (DBH; plants grown in the field), and total height (both experiments) were measured. The aboveground dry weight biomass for plants in the pot experiment was determined after destructive sampling at the end of the trial (after first growing season). The aboveground dry biomass of poplars in the field was estimated using a regression developed in a previous experiment. Specifically, the aboveground dry weight (DW) in grams was calculated using the following allometric function based on the DBH in mm:

$$DW = 1.1066 \cdot DBH^{2.0396}$$

Two green leaves from 4 trees randomly chosen in the field experiment for each experimental unit and two from each cutting in each pot were collected from the plants during the growing season and analysed as composite samples. From the stems harvested from each pot, the four bottom, middle and top 2 cm pieces were combined to respective composite samples. Leaf and stem samples were oven dried at 60°C to constant weight and ground to a fine powder using a ball mill (Retsch MM400 Mixer Mill, Retsch GmbH, 42781 Haan, Germany) with receptacle and balls made of agate. Total nitrogen contents of the ground material were measured by combustion using an elemental analyzer (NC 2500, CE Instruments Ltd, Wigan, UK). Total concentrations of other nutrients were determined by ICP-OES (Optima 7300 DV; Perkin Elmer, Waltham, MA, USA) of microwave digests with 8.3 M HNO3 / 0.6M HF (MWultraCLAV, MLS, Milestone Inc., Shelton, CT, USA).

Soil samples were collected at the end of 2018 to determine the nutrient supply and pH at the end of the experiments. In the field experiment, a cube of topsoil of 15x15x15 cm was excavated in approximately the centre of each experimental unit, whereas in the pot experiment, the complete top 15cm of soil in each pot were taken. The samples were sieved to 4 mm at field moisture and homogenized. Then one part was dried at 40°C and sieved to 2 mm. The latter part was used for determination of soil texture by the pipette method (Gee and Bauder, 1986), of soil pH (1:2 slurry in deionised water, 30 Min. equilibration), and extraction with 1M NH_4Cl (soil:extractant ratio 1:10, 1h). Exchangeable cations were measured in the latter extracts using ICP-OES (Optima 7300 DV; Perkin Elmer, Waltham, MA, USA). The field-moist part was used for the determination of ammonium and nitrate by extraction with 1 M KCl, as described by Shrestha et al. (2012), and of P fractions as follows. Resin exchangeable and microbial P were determined using the anion-exchange membrane method with and without hexanol fumigation as described by Bünemann et al. (2004). This was followed by extraction of the non-fumigated soil with 0.5M HCO_3 as described in the sequential extraction procedure by Tiessen and Moir (2006). Phosphate in all extracts was measured using Malachite Green (Ohno and Zibilske, 1991).

2.3 Growth, soil and nutrient data analysis

Data were analysed grouped, either for fertilized and unfertilized experimental units or pots with a given $CaCO_3$ level, or for experimental units or pots without or with fertilization, irrespective of $CaCO_3$ level. For each of the two experiments, a separate ANOVA was performed on the data testing for the effects of $CaCO_3$ addition and fertilization. The group means were tested for significant differences by means of post-hoc testing. In addition, to reveal common patterns in the two experiments, a linear mixed-effects (LME) model was applied to the combined growth and nutrient data from both experiments using the experiment type (field or pot) as random effect. A Type III Analysis of Variance Table with Satterthwaite's method was used to evaluate the effects of the factors $CaCO_3$ addition and fertilization (fixed effects) and their interaction. All statistical analyses were performed in R (R Development Core Team, 2020) using the lme4 package (Bates et al., 2015).

3. Results

3.1 pH and available nutrients in the topsoil

For a given combination of $CaCO_3$ level and experiment, fertilization did not affect soil pH (Tables 1 to 3). However, increasing $CaCO_3$ additions raised the pH in both experiments. Although the values did not differ among all individual CaCO3 levels (Tables 1 and 2), ANOVA revealed that, overall, the effects were highly significant in both experiments, as was the case when considering both experiments combined by LME modelling (Table 3). While the increase was almost linear in the pot experiment, a maximum was reached at C1500 in the field experiment. Furthermore, for a given treatment (except for the C1500 treatment), pH values were about 1 unit higher in the pot than in the field experiment.

The available nutrient concentrations in topsoil varied strongly within a given treatment and experiment (Tables 1, 2), and in addition differed partly between the two experiments for a given treatment (details of major nutrients and minor nutrients: Supplementary table). Despite this variability and these differences, irrespective of the experiment, $CaCO_3$ addition increased exchangeable Ca, while fertilization increased available P and exchangeable K concentrations. Although exchangeable Ca did not differ among all individual CaCO3 levels (Tables 1 and 2), ANOVA and LME modelling revealed that, overall, the effects were weakly significant in the field experiment, and highly significant in the pot experiment and when considering both experiments combined (Table 3). Fertilization significantly increased all measured P fractions in the field experiment and resin exchangeable P also in the pot experiment (Tables 1 to 3). Considering both experiments combined by LME modelling, the effects were highly significant except for microbial P (Table 3). The effect on exchangeable K was weakly significant in the pot experiment but significant in the field experiment and when considering both experiments combined (Tables 1 to 3). Furthermore, exchangeable Ca in both experiments exhibited the same linear relationship with pH (Fig. 2).

3.2 Plant nutrient status

As for nutrients in the soil, nutrient concentrations and ratios in leaves were highly variable within a given treatment and experiment, and in addition differed partly between the two experiments for a given treatment. In contrast to its significant effect on exchangeable Ca in the soil, $CaCO_3$ addition had no effects on foliar nutrient concentrations except for a slightly decreasing trend in foliar P (Tables 1, 2) and weakly significant effects on Ca (considering both experiments combined by LME modelling), and on the N:Ca ratio (pot experiment and both experiments combined, Table 4). Also, fertilization effects on the foliar nutrient concentrations differed distinctly from the effects on the soil. The most prominent and strongly significant effect was a decreased Mg concentration, and a respective increase in N:Mg (Table 4 and Supplementary Table). A similar effect was observed for the micro-nutrients B and Zn (Supplementary Table). In addition, fertilization led to a small but significant increase in N in the field experiment, to a small but significant decrease in Ca in the pot experiment, and to related significant effects on the N:P ratio in the field experiment and on the N:Ca ratio in the pot experiment (Tables 1, 2, and 4). All the latter effects were also significant when considering both experiments combined by LME modelling (Table 4).

In the case of the pot experiment, nutrient contents in the stem (concentrations x biomass) provided a different picture (Supplementary Table). Here, fertilization significantly increased the uptake of all measured nutrients ($P < 0.001$ for N, P, K, S, Ca; $P = 0.002$ for Mg), whereas $CaCO_3$ addition tended to increase Ca uptake only when combined with fertilization.

3.3 Plant growth

The three growth parameters we investigated (diameter, total height and biomass, as shown in Figs. 3, 4, and 5, respectively) were significantly increased by fertilization. For height, this effect was highly significant in both experiments, whereas for diameter and biomass this was the case in the pot experiment only, while the significance level was clearly lower in the field experiment (Table 5). This stronger fertilization effect in the pot than the field experiment could be related to the higher pH in the pot experiment, as is indicated by a positive correlation between the relative average fertilization effect per treatment/experiment combination with pH (Fig. 6).

When considering both experiments combined by LME modelling, the fertilization effects were highly significant for diameter and height, while the significance level was lower for biomass (Table 5). $CaCO_3$ addition had only weak increasing effects on diameter in the field experiment, and on biomass, when considering both experiments combined (Table 5).

4. Discussion

4.1 Growth response to calcium carbonate and NPKS fertilization

Taking the results on the growth response of poplar from our two experiments together, indicates that fertilization effects on growth were stronger when soil pH and exchangeable Ca values were above a certain level, thus confirming our hypothesis. Although the main effect of the fertilizer addition on soil was an increase in available P and K, indications from foliar nutrient analyses point rather to N as a limiting nutrient, as is argued in the following section. The overall stronger effect of fertilization in the pot than the field experiment can be explained by the higher starting pH and $CaCO_3$ containing irrigation solution in the pot experiment. Thus, in this experiment soil pH was at a sufficiently high level to make fertilization effective even without $CaCO_3$ addition. As is further elaborated in the following section, in this context not only soil pH is important but also the plant availability of Ca, thus suggesting an additional limiting effect of Ca on growth. Thus, our results not only support earlier findings on the effect of soil pH on the growth of poplar (Coudoun et al., 2006; Hjelm and Rytter, 2016), but also emphasize the importance of Ca in cell wall development and thus wood formation in poplar (Lautner et al., 2007; Baribault et al., 2012).

4.2 Effects of soil amendments on soil pH, available soil nutrients and plant nutritional status

In poplar plantations on acid soils, calcium carbonate is often used to increase soil pH (Böhlenius et al. 2020) and preferred to other forms of calcium containing materials such as e.g. gypsum, which does not increase soil pH. In our study, calcium carbonate increased soil pH in both experiments, irrespective of fertilization. The generally higher pH in the pot than the field experiment can be explained on one hand by the higher pH of the soil at the beginning of the experiment and by the carbonate containing irrigation water used in the pot experiment (as compared to water provided by rainfalls only in the field experiment with a pH below 7). Considering the optimum pH values for growth of poplar found to be between 6.0 and 6.5 (Dickmann et al., 2001), the consequence of the differences in starting and irrigation conditions was that in the pot experiment, already the lowest $CaCO_3$ dosage was sufficient to reach this pH level, whereas higher dosages were needed in the field experiment. Considering the overall consistent linear relationship between pH and exchangeable Ca in the two experiments (Fig. 2), the apparent failure of the highest dosage in the field experiment to increase the pH even higher is likely due to soil heterogeneity.

Overall, the effects of the soil amendments on available nutrient concentrations in the soil, i.e. an increase in Ca by $CaCO_3$ additions, and increase in K and P by fertilization, were not reflected by a respective increase in foliar concentrations. However, the results on nutrient contents in the stem in the pot experiment indicate that uptake of all nutrients was increased by fertilization. The decreased foliar concentrations of Mg and the micronutrients B and Zn in the fertilized treatments, nutrients added by neither amendment, indicate limited availability of these elements in the soil.

The foliar element concentrations and element ratios also provide some clues with respect to limiting nutrients. The positive effect of fertilization on the foliar N concentration and N:P ratio, while there was no effect on the mineral N concentrations in the soil, points to N limitation of plant growth. Under such conditions, plant uptake – and possibly microbial immobilization – would keep concentrations of available N in the soil low, irrespective of N addition. According to our hypothesis, the positive effect of $CaCO_3$ addition on plant nutrition could on one hand be attributed to an increase in soil pH above a value where sorption of anionic nutrients, in particular phosphate, becomes negligible with a positive effect on P availability, or to an increase in available Ca. Considering the close relationship between soil pH and exchangeable Ca, when combining the results of our experiments, the soil data do not allow us to distinguish between the two potential effects. However, the weak decreasing trend in foliar P concentrations with increasing $CaCO_3$ additions in both experiments may point to an additional role of Ca phosphate in controlling P availability, while the weakening effect on phosphate sorption should become larger with increasing $CaCO_3$ addition and thus pH. This further suggests that a positive effect of $CaCO_3$ addition on plant growth may at least partly be attributed to relieving Ca limitation.

Conclusion

This study evaluated the effects of the simultaneous application of calcium carbonate with NPKS fertilization on growth of poplar on acid soils based on two twin experiments in open field and in pots. Although the use of two different clones may explain some of the differences in plant growth and foliar nutrient concentrations between the two experiments, taken together the results indicate that fertilization effects on growth were stronger when soil pH and, related to this, exchangeable Ca were above a certain threshold. Thus, the results of this study on one hand emphasize the importance of adapting the level of $CaCO_3$ to the given soil and irrigation conditions, and on the other hand ask for further studies addressing the relative importance of elevated pH and improved Ca nutrition.

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Table 1. pH in topsoil, nutrient concentrations in topsoil (exchangeable Ca, K, SO4; mineral N, i.e. the sum of NH4-N and NO3-N; resin P) and green leaves (total element concentrations) of the field experiment; shown are Mean ± SD for fertilized and not fertilized plots with a given $CaCO_3$ addition (A), and for plots without or with fertilization, irrespective of $CaCO_3$ addition (B). Superscript letters (a, ab, b) refer to a post-hoc test we performed on the data.

	Topsoil concentrations [mg kg^{-1}]						Total foliar concentrations [g kg^{-1}]				
	pH topsoil	Ca_ex	N_min	P_resin	K_ex	SO4_ex_S	Ca	N	P	K	S
(A)											
C0	4.69^{b} ± 0.12	443^{b} ± 63	5.1^{a} ± 2.2	4.8^{a} ± 6.4	108^{a} ± 51	23.5^{a} ± 11.5	5.9^{a} ± 1.7	25.2^{a} ± 1.9	2.03^{a} ± 0.23	14.5^{a} ± 1.2	3.45^{a} ± 0.47
C500	4.87^{b} ± 0.19	493^{ab} ± 110	7.5^{a} ± 5.9	3.2^{a} ± 3.1	145^{a} ± 61	18.1^{a} ± 6.1	5.5^{a} ± 0.7	26.1^{a} ± 1.2	2.01^{a} ± 0.21	14.4^{a} ± 1.1	3.53^{a} ± 0.27
C1000	5.37^{ab} ± 0.59	656^{ab} ± 225	3.3^{a} ± 1.1	9.7^{a} ± 18.8	102^{a} ± 77	40.7^{a} ± 26.6	7.0^{a} ± 0.9	23.6^{a} ± 3.0	1.90^{a} ± 0.18	13.6^{a} ± 2.4	3.18^{a} ± 0.33
C1500	5.65^{a} ± 0.53	795^{a} ± 328	4.2^{a} ± 2.1	3.4^{a} ± 4.7	112^{a} ± 27	28.4^{a} ± 18.0	6.6^{a} ± 1.2	22.6^{a} ± 3.4	1.85^{a} ± 0.24	14.5^{a} ± 4.2	3.26^{a} ± 0.53
C2000	5.16^{ab} ± 0.40	712^{ab} ± 180	8.8^{a} ± 6.9	8.8^{a} ± 9.3	192^{a} ± 97	19.9^{a} ± 12.2	7.6^{a} ± 1.8	25.7^{a} ± 2.6	1.93^{a} ± 0.20	14.3^{a} ± 1.1	3.44^{a} ± 0.56
(B)											
without fert	5.23^{a} ± 0.52	592^{a} ± 168	4.7^{a} ± 4.8	1.4^{b} ± 1.5	92^{b} ± 39	21.6^{a} ± 9.9	6.6 ± 1.2	23.4^{b} ± 3.1	1.94^{a} ± 0.21	13.5^{a} ± 1.6	3.25^{a} ± 0.44
with fert.	5.06^{a} ± 0.51	648^{a} ± 285	6.9^{a} ± 4.0	10.5^{a} ± 12.3	172^{a} ± 74	30.6^{a} ± 22.0	6.5 ± 1.7	25.8^{a} ± 1.6	1.95^{a} ± 0.22	15.0^{a} ± 2.5	3.49^{a} ± 0.41

Table 2. pH in topsoil, nutrient concentrations in topsoil (exchangeable Ca, K, SO4; mineral N, i.e. the sum of NH4-N and NO3-N; resin P) and green leaves (total element concentrations) of the pot experiment; shown are Mean ± SD for fertilized and not fertilized pots together with a given $CaCO_3$ addition (A), and for pots without or with fertilization, irrespective of $CaCO_3$ addition (B). Superscript letters (a, ab, b) refer to a post-hoc test we performed on the data.

	Topsoil concentrations [mg kg^{-1}]						**Total foliar concentrations [g kg^{-1}]**				
	pH topsoil	**Ca_ex**	**N_min**	**P_resin**	**K_ex**	**SO4_ex_S**	**Ca**	**N**	**P**	**K**	**S**
A											
C0	5.57^{c} ± 0.22	880^{b} ± 205	1.2^{a} ± 0.7	5.6^{a} ± 6.8	69^{a} ± 14	2.7^{a} ± 1.2	13.4^{a} ± 2.1	21.9^{a} ± 5.2	1.74^{a} ± 0.18	9.8^{a} ± 2.7	3.51^{a} ± 1.12
C500	5.98^{bc} ± 0.44	1169^{b} ± 228	1.2^{a} ± 0.4	6.6^{a} ± 7.2	73^{a} ± 14	2.1^{a} ± 0.6	16.0^{a} ± 3.7	16.9^{a} ± 0.8	1.60^{a} ± 0.17	8.6^{a} ± 2.7	3.37^{a} ± 0.81
C1000	6.24^{b} ± 0.45	1350^{b} ± 502	0.9^{a} ± 0.7	6.8^{a} ± 6.8	72^{a} ± 11	2.8^{a} ± 1.3	15.6^{a} ± 2.9	17.8^{a} ± 3.8	1.60^{a} ± 0.23	8.6^{a} ± 2.1	3.30^{a} ± 0.52
C1500	6.40^{b} ± 0.37	1332^{b} ± 238	1.5^{a} ± 1.2	5.3^{a} ± 5.1	73^{a} ± 15	2.6^{a} ± 1.0	14.3^{a} ± 1.1	17.5^{a} ± 3.1	1.54^{a} ± 0.17	8.0^{a} ± 1.1	3.26^{a} ± 0.50
C2000	6.71^{a} ± 0.25	2073^{a} ± 709	1.2^{a} ± 0.5	7.0^{a} ± 7.6	73^{a} ± 12	3.0^{a} ± 1.3	13.2^{a} ± 2.1	17.3^{a} ± 2.2	1.52^{a} ± 0.21	8.0^{a} ± 1.6	3.23^{a} ± 0.72
B											
without fertilization	6.18^{a} ± 0.48	1317^{a} ± 653	1.3^{a} ± 0.8	1.9^{b} ± 0.9	67^{b} ± 11	2.3^{a} ± 1.0	15.8^{a} ± 2.4	17.6^{a} ± 3.1	1.61^{a} ± 0.18	8.0^{a} ± 1.6	3.57^{a} ± 0.48
with fertilization	6.18^{a} ± 0.56	1404^{a} ± 480	1.1^{a} ± 0.6	10.6^{a} ± 6.7	77^{a} ± 13	3.0^{a} ± 1.1	13.2^{b} ± 2.2	19.0^{a} ± 4.1	1.59^{a} ± 0.22	9.3^{a} ± 2.4	3.10^{a} ± 0.85

Table 3. Type III Analysis of Variance Table with Satterthwaite's method on soil pH and nutrient concentrations in the topsoil (exchangeable Ca, K, SO4, Mg; mineral N, i.e. the sum of NH4-N and NO3-N; hydrogencarbonate extractable P, resin P, microbial P), considering the data of both field and pot experiments combined (mixed effects model). In addition, probability values are given for ANOVAs run on the data from the individual experiments. Statistically significant effects are denoted with asterisks according to the following criteria: 0 ≤ *** < 0.001 ≤ ** < 0.01 ≤ * < 0.05

MIXED EFFECTS MODEL									**ANOVA ON FIELD DATA**	**ANOVA ON POT DATA**
Soil parameter	**R2 Fixed terms**	**R2 Model**	**Source of variation**	**DF**	**Sum Sq**	**Mean Sq**	**F.value**	**Pr(>F)**	**Pr(>F)**	**Pr(>F)**
pH	0.145	0.801	Calcium carbonate	4	11.1	2.8	17.212	0.000***	<0.001***	<0.001***
			Fertilization	1	0.3	0.3	1.629	0.205	0.092	0.972
			Calc x Fert	4	0.2	0.1	0.348	0.845	0.779	0.779
Ca_ex	0.086	0.747	Calcium carbonate	4	3758253.3	939563.3	7.937	<0.001***	0.018*	<0.001***
			Fertilization	1	69041.2	69041.2	0.583	0.447	0.518	0.537
			Calc x Fert	4	165772.1	41443.1	0.350	0.843	0.764	0.778
N_min	0.106	0.502	Calcium carbonate	4	137.6	34.4	4.057	0.005**	0.003**	0.486
			Fertilization	1	27.8	27.8	3.282	0.073	0.041*	0.376
			Calc x Fert	4	10.8	2.7	0.318	0.856	0.833	0.122
P_HCO_3	0.179	0.209	Calcium carbonate	4	483.1	120.8	1.200	0.317	0.196	0.949
			Fertilization	1	1668.2	1668.2	16.573	<0.001***	0.001**	0.051
			Calc x Fert	4	99.2	24.8	0.246	0.911	0.850	0.707
P_resin	0.247	0.279	Calcium carbonate	4	137.0	34.3	0.893	0.472	0.563	0.952
			Fertilization	1	1057.4	1057.4	27.565	<0.001***	0.008**	<0.001***
			Calc x Fert	4	107.9	26.9	0.703	0.591	0.709	0.851
P_mic	0.044	0.652	Calcium carbonate	4	18.1	4.5	0.247	0.911	0.308	0.733
			Fertilization	1	78.8	78.8	4.292	0.041*	0.042*	0.376
			Calc x Fert	4	132.7	33.2	1.808	0.134	0.053	0.128
K_ex	0.182	0.410	Calcium carbonate	4	25625.4	6406.3	3.304	0.014*	0.010*	0.963
			Fertilization	1	21406.9	21406.9	11.042	0.001**	0.003**	0.022*
			Calc x Fert	4	12133.2	3033.3	1.565	0.191	0.139	0.632
SO_4_ex	0.072	0.422	Calcium carbonate	4	1599.5	399.9	2.565	0.044*	0.044*	0.544
			Fertilization	1	62.7	62.7	0.402	0.527	0.585	0.088
			Calc x Fert	4	654.2	163.5	1.049	0.386	0.372	0.819
Mg_ex	0.023	0.745	Calcium carbonate	4	1164.6	291.2	1.010	0.407	0.798	0.393
			Fertilization	1	874.2	874.2	3.033	0.085	0.960	0.003**
			Calc x Fert	4	534.9	133.8	0.464	0.761	0.786	0.648

Table 4. Type III Analysis of Variance Table with Satterthwaite's method on foliar nutrient concentrations and ratios, considering the data of both field and pot experiments combined (mixed effects model). In addition, probability values are given for ANOVAs run on the data from the individual experiments. Statistically significant effects are denoted with asterisks according to the following criteria: 0 ≤ *** < 0.001 ≤ ** < 0.01 ≤ * < 0.05

MIXED EFFECTS MODEL									ANOVA ON FIELD DATA	ANOVA ON POT DATA
Plant parameter	**R2 Fixed terms**	**R2 Model**	**Source of variation**	**DF**	**Sum Sq**	**Mean Sq**	**F.value**	**Pr(>F)**	**Pr(>F)**	**Pr(>F)**
Ca	0.024	0.884	Calcium carbonate	4	16445046.4	4111261.6	0.958	0.438	0.131	0.164
			Fertilization	1	24847607.1	24847607.1	5.800	0.019*	0.967	0.006**
			Calc x Fert	4	10242572.3	2560643.1	0.598	0.666	0.931	0.612
N	0.104	0.739	Calcium carbonate	4	80.9	20.2	2.459	0.058	0.040*	0.116
			Fertilization	1	53.7	53.7	6.529	0.014*	0.004**	0.288
			Calc x Fert	4	58.5	14.6	1.775	0.149	0.109	0.593
P	0.084	0.636	Calcium carbonate	4	258659.5	64664.9	1.721	0.160	0.596	0.426
			Fertilization	1	267.9	267.9	0.007	0.933	0.877	0.781
			Calc x Fert	4	250587.2	62646.8	1.667	0.173	0.450	0.676
K	0.036	0.784	Calcium carbonate	4	9422119.1	2355529.8	0.517	0.723	0.947	0.533
			Fertilization	1	28407678.9	28407678.9	6.239	0.016*	0.102	0.095
			Calc x Fert	4	6496424.4	1624106.1	0.357	0.838	0.590	0.414
S	0.096	0.096	Calcium carbonate	4	572242.9	143060.7	0.385	0.818	0.609	0.959
			Fertilization	1	211050.0	211050.0	0.568	0.455	0.140	0.081
			Calc x Fert	4	1190215.9	297554.0	0.801	0.530	0.346	0.255
Mg	0.322	0.322	Calcium carbonate	4	2970452.5	742613.1	1.184	0.329	0.624	0.517
			Fertilization	1	11678678.6	11678678.6	18.623	<0.001***	0.007**	0.006**
			Calc x Fert	4	2890114.6	722528.7	1.152	0.343	0.820	0.223
N:Ca	0.036	0.875	Calcium carbonate	4	6.2	1.6	2.960	0.029*	0.072	0.010*
			Fertilization	1	2.1	2.1	3.969	0.052	0.245	0.002**
			Calc x Fert	4	0.7	0.2	0.345	0.846	0.907	0.753
N:P	0.163	0.432	Calcium carbonate	4	7.4	1.7	0.961	0.437	0.560	0.269
			Fertilization	1	18.9	18.7	10.262	0.002**	0.020*	0.078
			Calc x Fert	4	5.1	1.2	0.695	0.599	0.915	0.783
N:K	0.029	0.377	Calcium carbonate	4	0.1	0.1	0.110	0.978	0.784	0.987
			Fertilization	1	0.1	0.1	0.308	0.581	0.837	0.497
			Calc x Fert	4	0.3	0.1	0.505	0.732	0.841	0.644
N:Mg	0.217	0.638	Calcium carbonate	4	9.8	2.4	2.406	0.062	0.144	0.220
			Fertilization	1	25.4	25.3	24.946	<0.001***	0.001**	0.006**
			Calc x Fert	4	0.8	0.2	0.207	0.933	0.801	0.977

Table 5. Type III Analysis of Variance Table with Satterthwaite's method on the three growth parameters, considering the data of both field and pot experiments combined (mixed effects model). In addition, probability values are given for ANOVAs run on the data from the individual experiments. Statistically significant effects are denoted with asterisks according to the following criteria: 0 ≤ *** < 0.001 ≤ ** < 0.01 ≤ * < 0.05-

MIXED EFFECTS MODEL								ANOVA ON FIELD DATA	ANOVA ON POT DATA
Parameter	**Fixed-effect**	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**	**Pr(>F)**	**Pr(>F)**
Diameter	Calcium carbonate	1.06	0.265	4	537	2.30	0.058	0.0398*	0.6410
	Fertilization	1.62	1.62	1	537	14.0	<0.001***	0.0137*	<0.001***
	Calc x Fert	0.163	0.041	4	537	0.35	0.84	0.8506	0.2445
Height	Calcium carbonate	1.06	0.264	4	537	2.29	0.059	0.0995	0.3464
	Fertilization	1.62	1.62	1	537	14.0	<0.001***	<0.001***	<0.001***
	Calc x Fert	0.165	0.041	4	537	0.36	0.84	0.9668	0.0596
Biomass	Calcium carbonate	10.4	2.599	4	538	2.59	0.036*	0.0930	0.7453
	Fertilization	8.7	8.7	1	538	8.69	0.0034**	0.0023**	<0.001***
	Calc x Fert	1.94	0.486	4	538	0.48	0.75	0.9229	0.1728

Fig. 1 Spatial layout of the field experiment (split-plot design)

Fig. 2. Exchangeable Ca vs. pH in the topsoil of the two experiments.

Fig. 3 Boxplots of measured tree diameters (in mm, field: at breast height; pot: 20cm above collar) in the two experiments and letters indicating significant differences according to the post-hoc test. Blue boxplots show the variabilities for given calcium carbonate levels irrespective of fertilization; conversely red boxplots show the variabilities for fertilised and non-fertilised samples irrespective of level of added calcium carbonate.

Fig. 4 Boxplots of measured tree heights (in cm) in the two experiments and letters indicating significant differences according to the post-hoc test. Blue boxplots show the variabilities for given calcium carbonate levels irrespective of fertilization; conversely red boxplots show the variabilities for fertilised and non-fertilised samples without irrespective of level of added calcium carbonate.

Fig. 5. Boxplots of measured total tree biomass (grams dry weight) in the two experiments and letters indicating significant differences according to the post-hoc test. Blue boxplots show the variabilities for given calcium carbonate levels irrespective of fertilization; conversely red boxplots show the variabilities for fertilised and non-fertilised samples irrespective of level of added calcium carbonate.

Fig.6. Average fertilizer effect vs. topsoil pH in the two experiments. The fertilizer effect for a given $CaCO_3$ addition was calculated by dividing the median plant biomass of the respective fertilized treatment by the median plant biomass of the respective unfertilized treatment, followed by multiplication with 100.

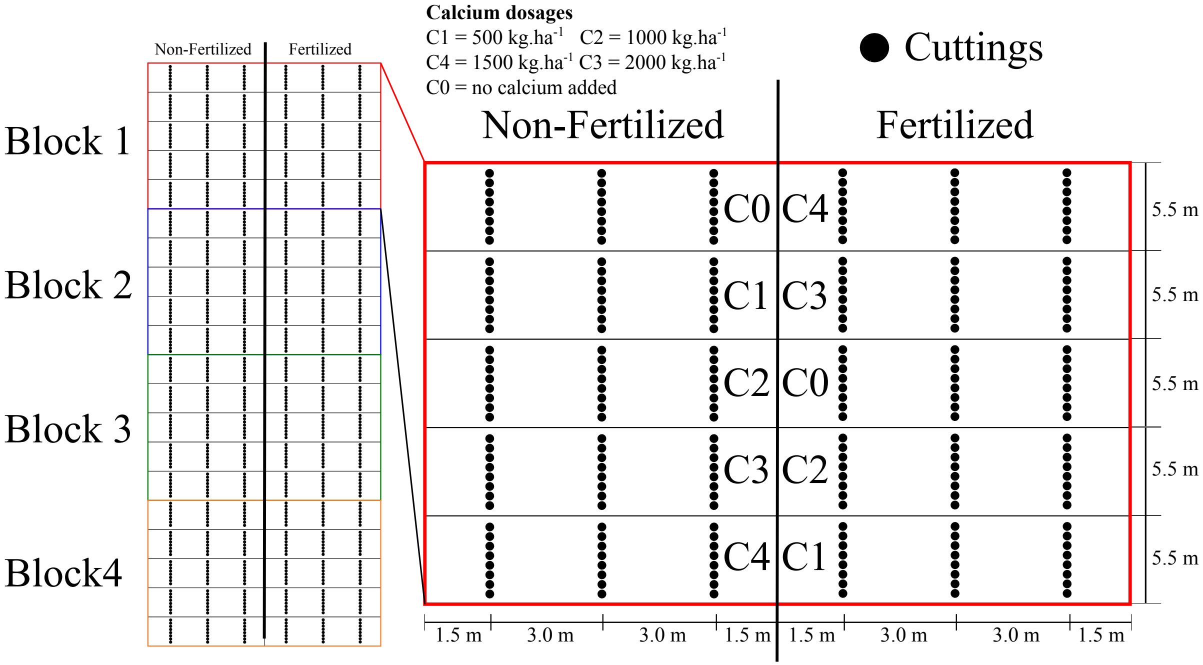

Calcium dosages
C1 = 500 kg.ha-1 C2 = 1000 kg.ha-1
C4 = 1500 kg.ha-1 C3 = 2000 kg.ha-1
C0 = no calcium added
● Cuttings
Non-Fertilized
Fertilized
Block 1
Block 2
Block 3
Block4
C0 C4
C1 C3
C2 C0
C3 C2
C4 C1
5.5 m
5.5 m
5.5 m
5.5 m
5.5 m
1.5 m
3.0 m
3.0 m
1.5 m
1.5 m
3.0 m
3.0 m
1.5 m

Fig. 1

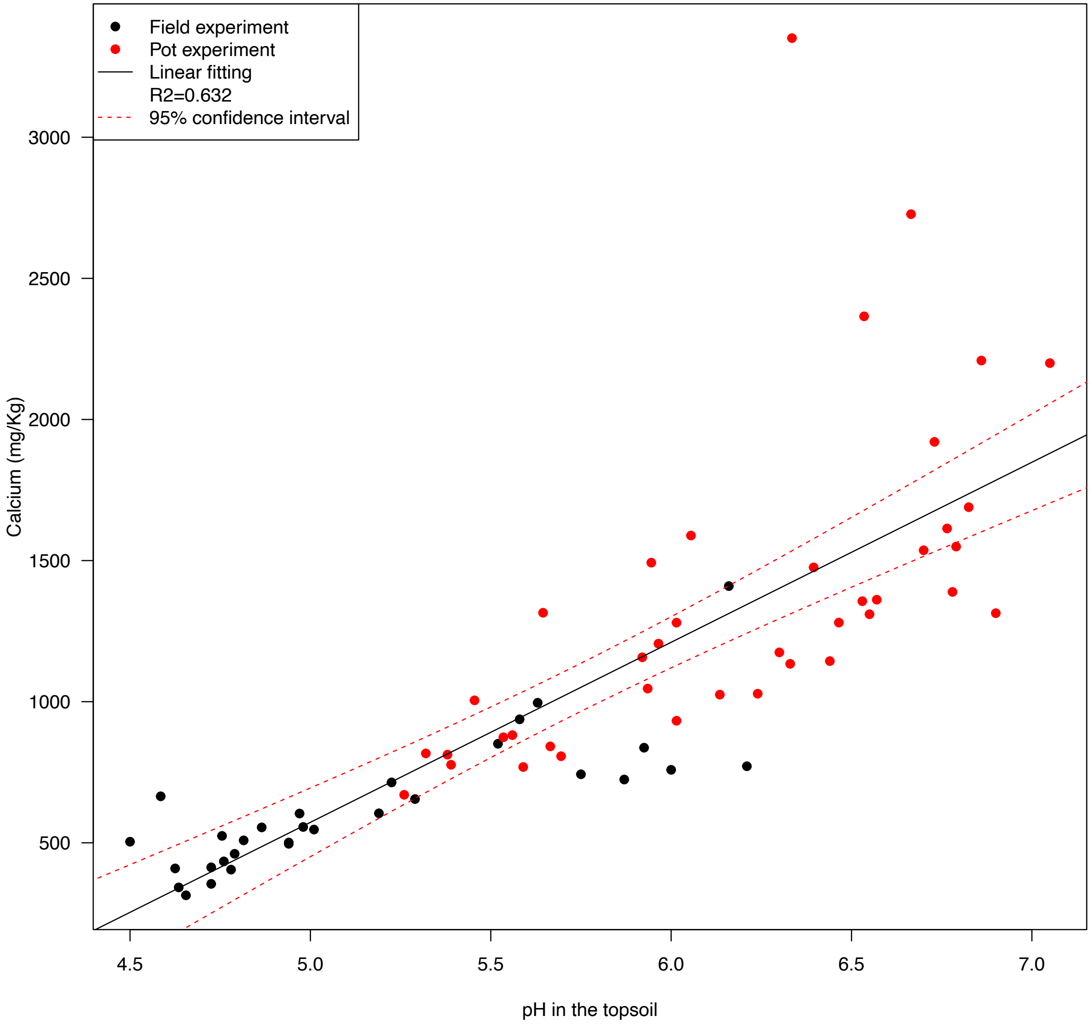

Field experiment
Pot experiment
Linear fitting
R2=0.632
95% confidence interval
Calcium (mg/Kg)
3000
2500
2000
1500
1000
500
4.5
5.0
5.5
6.0
6.5
7.0
pH in the topsoil

Fig. 2

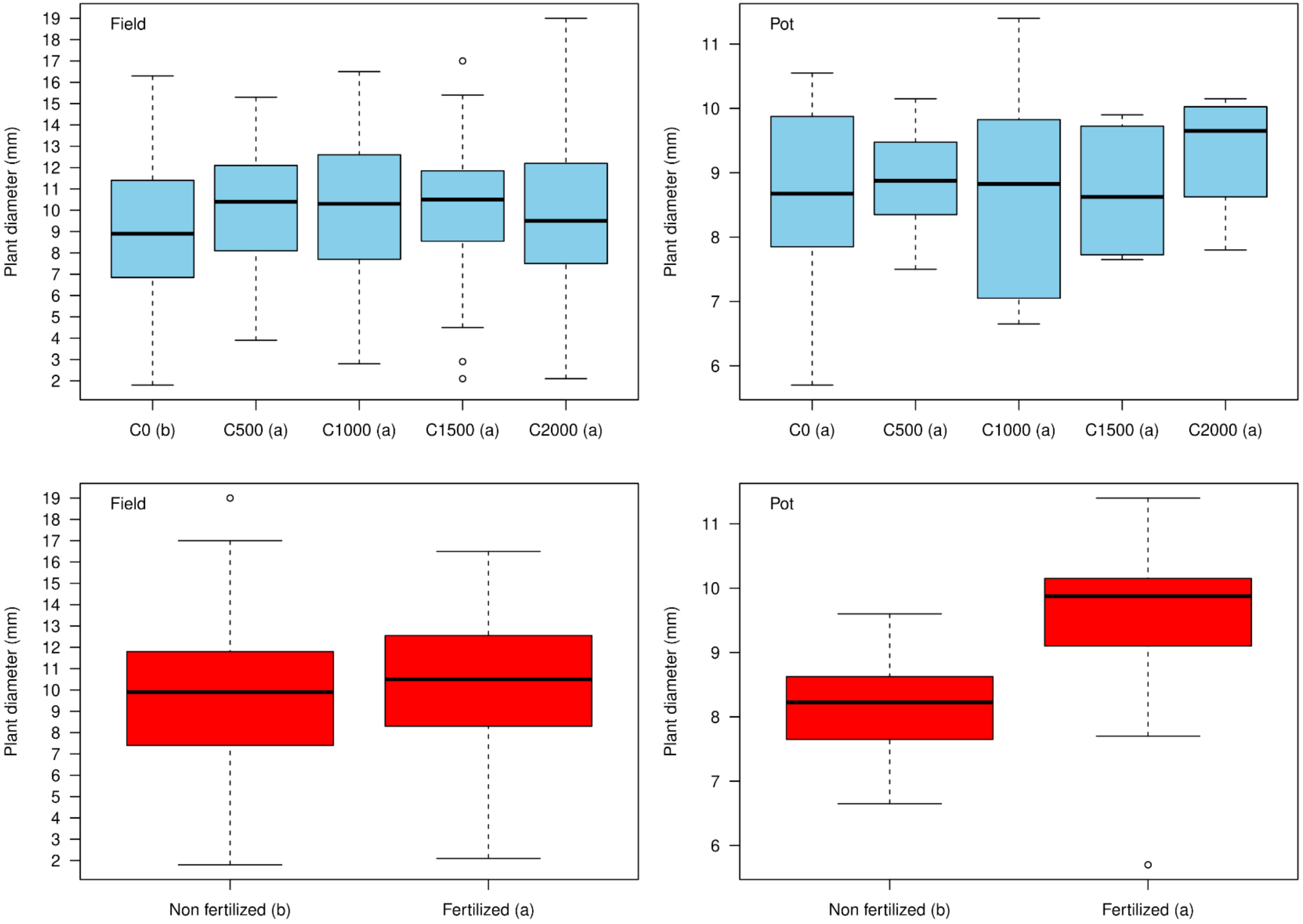

Field
Plant diameter (mm)
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
C0 (b)
C500 (a)
C1000 (a)
C1500 (a)
C2000 (a)
Pot
Plant diameter (mm)
11
10
9
8
7
6
C0 (a)
C500 (a)
C1000 (a)
C1500 (a)
C2000 (a)
Field
Plant diameter (mm)
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
Non fertilized (b)
Fertilized (a)
Pot
Plant diameter (mm)
11
10
9
8
7
6
Non fertilized (b)
Fertilized (a)

Fig 3

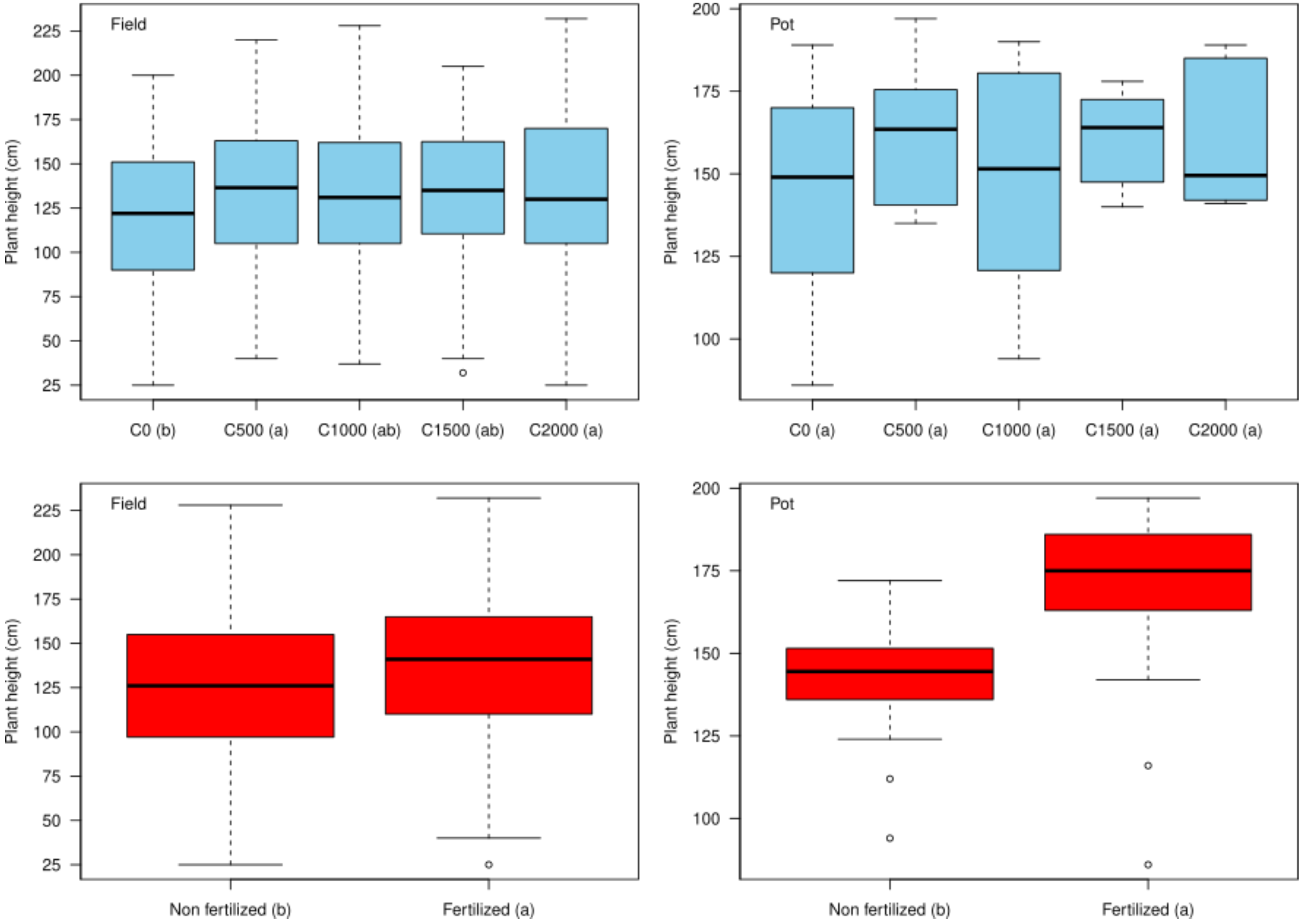

Field
Plant height (cm)
225
200
175
150
125
100
75
50
25
C0 (b)
C500 (a)
C1000 (ab)
C1500 (ab)
C2000 (a)
Pot
Plant height (cm)
200
175
150
125
100
C0 (a)
C500 (a)
C1000 (a)
C1500 (a)
C2000 (a)
Field
Plant height (cm)
225
200
175
150
125
100
75
50
25
Non fertilized (b)
Fertilized (a)
Pot
Plant height (cm)
200
175
150
125
100
Non fertilized (b)
Fertilized (a)

Fig. 4

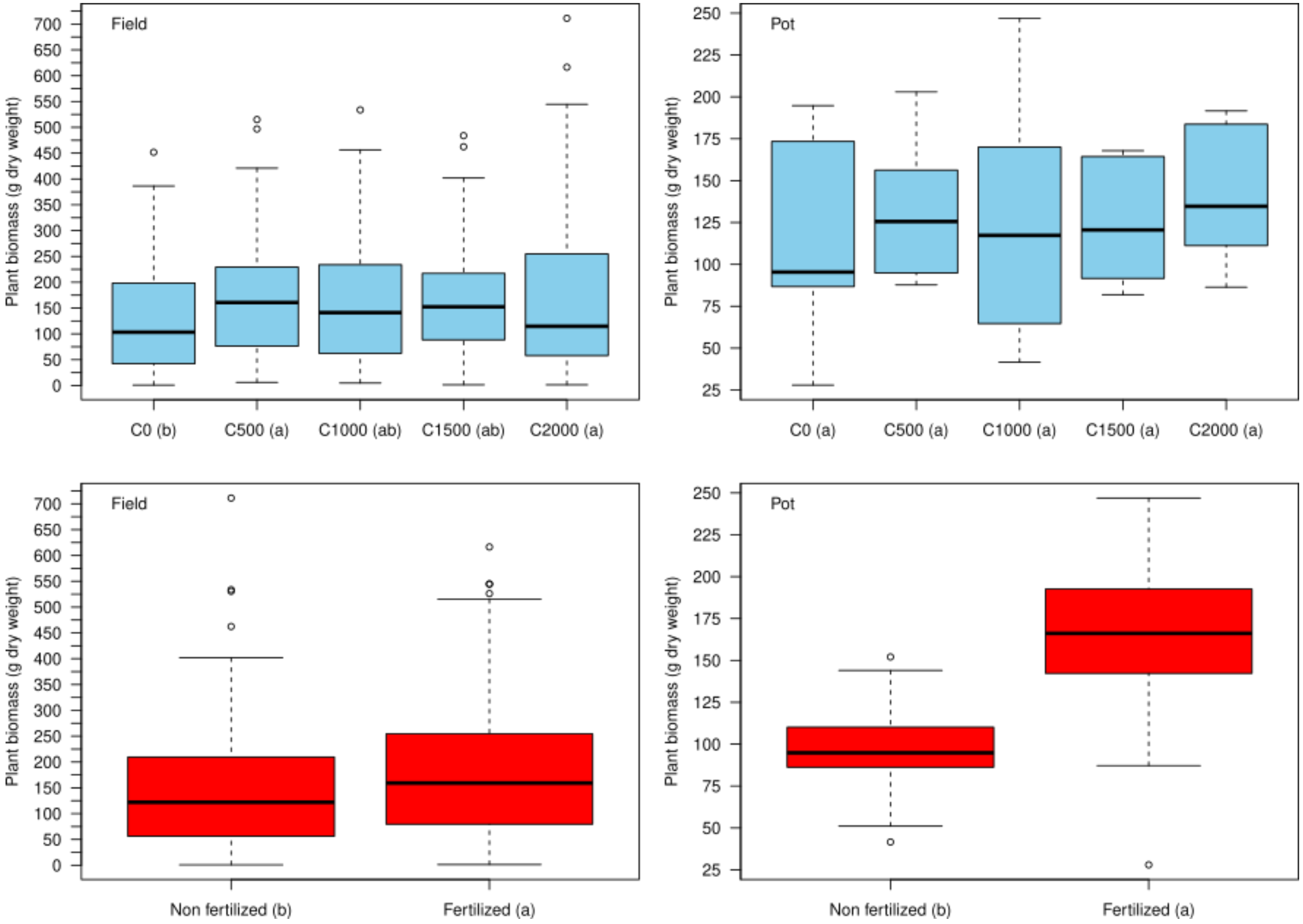
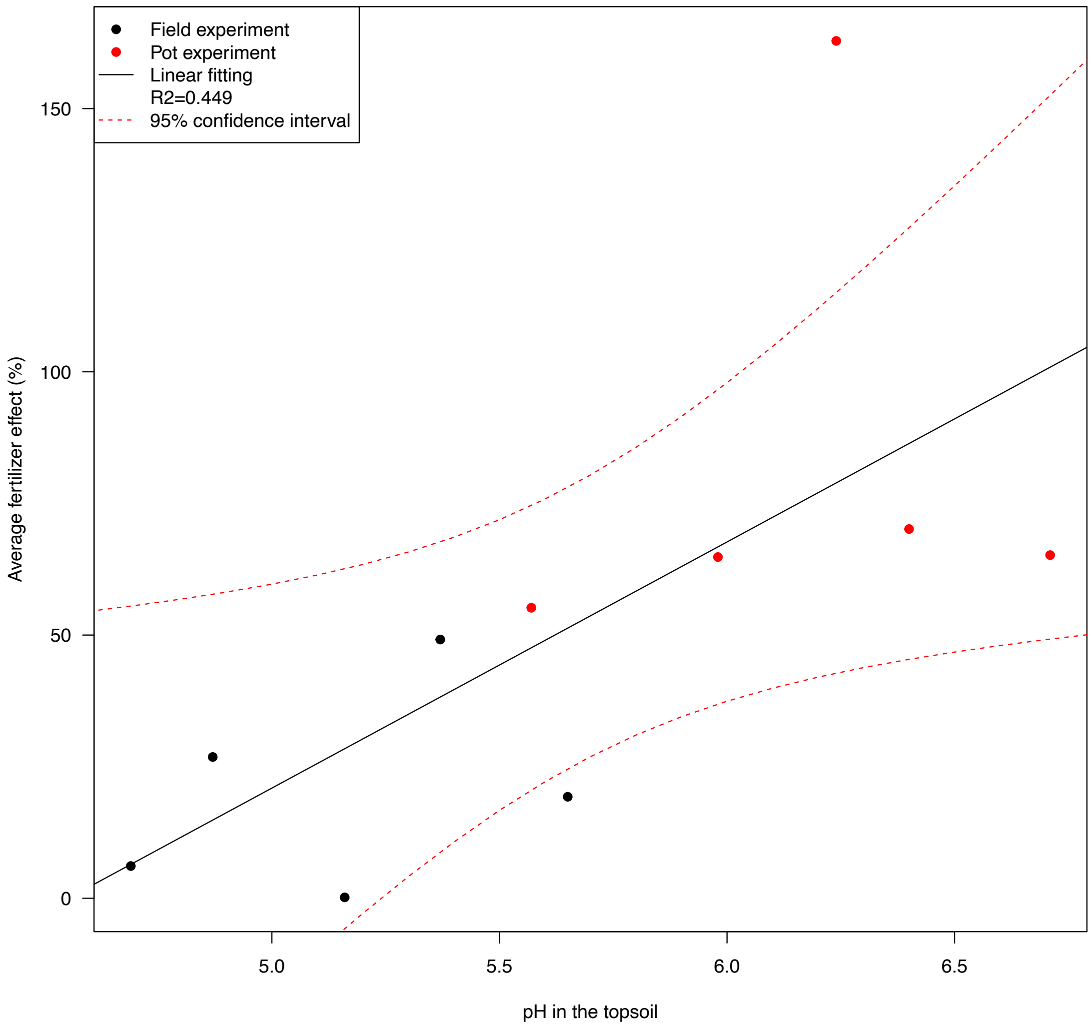

Field
Pot
Plant biomass (g dry weight)
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
25
75
125
175
225
C0 (b)
C500 (a)
C1000 (ab)
C1500 (ab)
C2000 (a)
C0 (a)
C500 (a)
C1000 (a)
C1500 (a)
C2000 (a)
Non fertilized (b)
Fertilized (a)

Fig. 5



● Field experiment
● Pot experiment
Linear fitting
R2=0.449
95% confidence interval
Average fertilizer effect (%)
0
50
100
150
5.0
5.5
6.0
6.5
pH in the topsoil

Fig. 6