

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

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17 **Abstract:** The cultivation of *Populus* spp. on acid soils is difficult mainly due to low nutrient  
18 availability, limiting the distribution and use of this marketable tree species. In this paper we  
19 report the results of two experiments, in which a granulated highly reactive micronized calcium  
20 carbonate (CaCO<sub>3</sub>) was tested at increasing levels to improve the effect of NPKS fertilization  
21 on poplar growth. Twin field and pot experiments were carried out in 2017 using two different  
22 poplar clones, both of which are often used in Italy. In addition to analysing the data from the  
23 two experiments separately, common patterns were evaluated using a mixed-effect model with  
24 CaCO<sub>3</sub> level and fertilization as fixed effects, and the experiment type as random effect. Growth  
25 was assessed in terms of total height, diameter and biomass. Taken together, the results from  
26 the two experiments showed that fertilization led to enhanced growth of poplar, but this effect

27 was stronger when soil conditions in terms of pH and exchangeable Ca were at a sufficiently  
28 high level. Available nutrient concentrations in the soil and foliar nutrient concentrations in the  
29 plant suggested co-limitation of poplar growth by N and Ca. In conclusion, the results of this  
30 study on one hand emphasize the importance of adapting the level of CaCO<sub>3</sub> to the given soil  
31 conditions, and on the other hand ask for further studies addressing the relative importance of  
32 elevated pH and improved Ca nutrition.

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

36

## 37 **1. Introduction**

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

52 Climate and soil characteristics are known to influence the plant availability of soil  
53 nutrients and thus the efficacy of fertilization (Doty et al., 2016; Rennenberg et al., 2010). Soil  
54 pH is a major factor known to influence nutrient availability (Böhlenius et al., 2020). In  
55 extremely acid or alkaline soils, nutrient availability is often low, and as a consequence, plant  
56 growth reduced (Hjelm and Rytter, 2016). At pH lower than 5- 5.5, the availability of many  
57 nutrients including the major nutrients N, P, K, Ca, and Mg is limited. In particular, the fixation  
58 of nutrients occurring in anionic form such as P at low pH and the respective limiting effect on  
59 fertilization have been demonstrated, e.g. by Fernández and Hoefl (2009). As a consequence,  
60 fertilization of acidic soils often fails to achieve its purpose and may in addition lead to  
61 environmental problems due to increased risk associated with the leaching of cationic nutrients  
62 (Beaudoin et al., 2005). In this framework, liming is a common agronomic practice performed  
63 on soils with low pH values to improve nutrient availability, as well as to address the issues of  
64 nutrient fixation and Al toxicity. For this purpose, different calcium (Ca) containing compounds  
65 can be used such as  $\text{CaCO}_3$ ,  $\text{Ca(OH)}_2$ ,  $\text{CaMg(CO}_3\text{)}$ ,  $\text{CaO}$  and all have been shown to be able to  
66 raise the pH and to mobilize some nutrients (Holland et al., 2018). If applied correctly in terms  
67 of quantity and time, this soil amendment has multiple advantages, including mobilization of  
68 anionic nutrients and thus improving their plant uptake, decreasing leaching losses of cationic  
69 nutrients, and enhancing soil microbial activity. While some studies on growth response of  
70 fertilized poplar seedlings have proven that the uptake of added nutrients can be increased by  
71 raising soil pH (Foster and Bhatti, 2006; Han et al., 2016), other studies have not shown any  
72 effect of liming on growth of forest trees (Reid and Watmough, 2014). An important additional  
73 effect can be improved Ca nutrition of the plants, since exchangeable Ca is inherently low in  
74 acid soils (Foster and Bhatti, 2006; De Vries and Posch, 2011), and reductions in exchangeable  
75 Ca have been associated with growth declines in several woody species (Schmidt et al., 2015).  
76 Calcium is an important macronutrient required by trees, in particular by poplar. For this  
77 species, Lautner et al. (2007) demonstrated that Ca acts as an important regulator in many

78 processes related to growth and wood formation and responses to environmental stresses.  
79 Nevertheless, the appropriate levels of Ca dosage in forest plantations and tree farming are still  
80 unknown for many tree species (Grover et al., 2021).  
81 In this study, we address the question how different combinations of liming and fertilization  
82 affect the growth of hybrid poplar trees and how this is related to pH and the availability of Ca  
83 and other major nutrients. Considering the scientific evidence for the importance of calcium  
84 addition on wood formation discussed above and the contradictory results with respect to  
85 growth response following liming, we investigated the effect of calcium addition in form of  
86 calcium carbonate ( $\text{CaCO}_3$ ) in combination with standard NPKS fertilization on growth and  
87 nutrient uptake of poplar in acid soils. Specifically, based on results from a field and pot  
88 experiment, we test the hypothesis that the growth of poplar on acid soils can only be increased  
89 by fertilization if soil pH is raised sufficiently by  $\text{CaCO}_3$  to (i) minimize the abundance of  
90 anionic exchange sites thus increasing particularly the availability of P, and (ii) to increase the  
91 concentration of exchangeable Ca.

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93

## 94 **2. Materials and Methods**

### 95 *2.1 Experimental design*

96 In early April of 2017, two twin experiments, both with a randomised split-plot design  
97 comprising 4 replicates, were realised in Northern Italy. Two poplar clones of recent wide  
98 commercial use in Italy were employed. Cuttings with an average diameter of approximately  
99 1.5 cm and a length of about 22 cm were collected from a nursery in Casale Monferrato in late  
100 2016. In the first experiment cuttings of the ‘Orion’ clone (*Populus ×canadensis*) were planted  
101 in open field at “Mezzomerico” (MER; 45°38'09.1"N 8°35'18.8"E). This trial covered a total  
102 area of 2,000 m<sup>2</sup> with 4 blocks of 27.5 m x 18 m (Fig. 1). Inside each block, 10 experimental  
103 units of 9 × 5.5 m were defined, one for each fertilization x calcium carbonate treatment (see

104 below). For practical reasons, in each block the same sub-blocks of 5 units were fertilized or  
105 unfertilized, while the 5 calcium carbonate levels were assigned randomly within each sub-  
106 block (Fig. 1). In each experimental unit, 24 cuttings were planted in 3 rows. The spacing was  
107 3 m between planting rows and 0.65 m between individual plants within a given row. This  
108 spatial design is the typical layout used in Italy for a Short Rotation Coppice plantation. An  
109 additional spacing of 1 m between the two plants in a given planting row belonging to different  
110 experimental units was added to ensure the absence of edge effects between treatments. A  
111 composite soil sample from the field was composed of 4 % coarse sand, 22% fine sand, 30%  
112 coarse silt, 26% fine silt and 10% clay (silty loam), and the pH (in H<sub>2</sub>O) without amendment  
113 was 4.4.

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

124 The fertilization consisted of nitrogen (N), phosphorus (P), potassium (K) and sulphur (S)  
125 (NPK(S) 15.15.15.(5)), i.e. 15% N (4% NH<sub>4</sub>, 11% Urea), 15% P<sub>2</sub>O<sub>5</sub> (5% soluble in ammonium  
126 citrate and water, 10% soluble in mineral acids), 15% K<sub>2</sub>O soluble in water and 5% of SO<sub>3</sub>  
127 soluble in water, and was applied at a rate of 100 kg ha<sup>-1</sup> each of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O and 33 kg  
128 ha<sup>-1</sup> of SO<sub>3</sub>. This type of fertilization is the most common practice in Italy. The 5 calcium  
129 carbonate levels were 0, 500, 1000, 1500, and 2000 kg ha<sup>-1</sup>, herein referred to as C0, C500,

130 C1000, C1500 and C2000, respectively. The calcium source used in this study was Omya  
131 Calciprill®, a granulated highly reactive micronized calcium carbonate (CaCO<sub>3</sub>). The granules  
132 had an average size of 2-6 mm and consisted of ≥ 91% chalk present in form of micronized  
133 particles with a weighted median particle size of d<sub>50</sub> = 4.5 μm. Calcium carbonate amendment  
134 and fertilizer were spread manually; the former was distributed only during establishment of  
135 the trial, while fertilizer was spread at the end of May (late Spring) of the first and second year  
136 of growth for the field experiment. In the pot experiments, irrigation combined with insecticide  
137 treatment was applied twice per week. The pH of the irrigation water was 7.74, as measured at  
138 the beginning of the experiment. In the open field, weeds were controlled using herbicides after  
139 planting and rotovators during summer. The field experiment was not irrigated but received  
140 only rainfalls.

141

## 142 *2.2 Data collection*

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

150

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

151 Two green leaves from 4 trees randomly chosen in the field experiment for each  
152 experimental unit and two from each cutting in each pot were collected from the plants during  
153 the growing season and analysed as composite samples. From the stems harvested from each  
154 pot, the four bottom, middle and top 2 cm pieces were combined to respective composite  
155 samples. Leaf and stem samples were oven dried at 60°C to constant weight and ground to a

156 fine powder using a ball mill (Retsch MM400 Mixer Mill, Retsch GmbH, 42781 Haan,  
157 Germany) with receptacle and balls made of agate. Total nitrogen contents of the ground  
158 material were measured by combustion using an elemental analyzer (NC 2500, CE Instruments  
159 Ltd, Wigan, UK). Total concentrations of other nutrients were determined by ICP-OES (Optima  
160 7300 DV; Perkin Elmer, Waltham, MA, USA) of microwave digests with 8.3 M HNO<sub>3</sub> / 0.6M  
161 HF (MWUltraCLAV, MLS, Milestone Inc., Shelton, CT, USA).

162 Soil samples were collected at the end of 2018 to determine the nutrient supply and pH at  
163 the end of the experiments. In the field experiment, a cube of topsoil of 15x15x15 cm was  
164 excavated in approximately the centre of each experimental unit, whereas in the pot experiment,  
165 the complete top 15cm of soil in each pot were taken. The samples were sieved to 4 mm at field  
166 moisture and homogenized. Then one part was dried at 40°C and sieved to 2 mm. The latter  
167 part was used for determination of soil texture by the pipette method (Gee and Bauder, 1986),  
168 of soil pH (1:2 slurry in deionised water, 30 Min. equilibration), and extraction with 1M NH<sub>4</sub>Cl  
169 (soil:extractant ratio 1:10, 1h). Exchangeable cations were measured in the latter extracts using  
170 ICP-OES (Optima 7300 DV; Perkin Elmer, Waltham, MA, USA). The field-moist part was  
171 used for the determination of ammonium and nitrate by extraction with 1 M KCl, as described  
172 by Shrestha et al. (2012), and of P fractions as follows. Resin exchangeable and microbial P  
173 were determined using the anion-exchange membrane method with and without hexanol  
174 fumigation as described by Bünemann et al. (2004). This was followed by extraction of the non-  
175 fumigated soil with 0.5M HCO<sub>3</sub> as described in the sequential extraction procedure by Tiessen  
176 and Moir (2006). Phosphate in all extracts was measured using Malachite Green (Ohno and  
177 Zibilske, 1991).

178

### 179 *2.3 Growth, soil and nutrient data analysis*

180 Data were analysed grouped, either for fertilized and unfertilized experimental units or pots  
181 with a given CaCO<sub>3</sub> level, or for experimental units or pots without or with fertilization,

182 irrespective of CaCO<sub>3</sub> level. For each of the two experiments, a separate ANOVA was  
183 performed on the data testing for the effects of CaCO<sub>3</sub> addition and fertilization. The group  
184 means were tested for significant differences by means of post-hoc testing. In addition, to  
185 reveal common patterns in the two experiments, a linear mixed-effects (LME) model was  
186 applied to the combined growth and nutrient data from both experiments using the experiment  
187 type (field or pot) as random effect. A Type III Analysis of Variance Table with Satterthwaite's  
188 method was used to evaluate the effects of the factors CaCO<sub>3</sub> addition and fertilization (fixed  
189 effects) and their interaction. All statistical analyses were performed in R (R Development Core  
190 Team, 2020) using the lme4 package (Bates et al., 2015).

191

### 192 **3. Results**

#### 193 *3.1 pH and available nutrients in the topsoil*

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

202 The available nutrient concentrations in topsoil varied strongly within a given treatment  
203 and experiment (Tables 1, 2), and in addition differed partly between the two experiments for  
204 a given treatment (details of major nutrients and minor nutrients: Supplementary table). Despite  
205 this variability and these differences, irrespective of the experiment, CaCO<sub>3</sub> addition increased  
206 exchangeable Ca, while fertilization increased available P and exchangeable K concentrations.  
207 Although exchangeable Ca did not differ among all individual CaCO<sub>3</sub> levels (Tables 1 and 2),



208 ANOVA and LME modelling revealed that, overall, the effects were weakly significant in the  
209 field experiment, and highly significant in the pot experiment and when considering both  
210 experiments combined (Table 3). Fertilization significantly increased all measured P fractions  
211 in the field experiment and resin exchangeable P also in the pot experiment (Tables 1 to 3).  
212 Considering both experiments combined by LME modelling, the effects were highly significant  
213 except for microbial P (Table 3). The effect on exchangeable K was weakly significant in the  
214 pot experiment but significant in the field experiment and when considering both experiments  
215 combined (Tables 1 to 3). Furthermore, exchangeable Ca in both experiments exhibited the  
216 same linear relationship with pH (Fig. 2).

217

### 218 *3.2 Plant nutrient status*

219 As for nutrients in the soil, nutrient concentrations and ratios in leaves were highly variable  
220 within a given treatment and experiment, and in addition differed partly between the two  
221 experiments for a given treatment. In contrast to its significant effect on exchangeable Ca in the  
222 soil, CaCO<sub>3</sub> addition had no effects on foliar nutrient concentrations except for a slightly  
223 decreasing trend in foliar P (Tables 1, 2) and weakly significant effects on Ca (considering both  
224 experiments combined by LME modelling), and on the N:Ca ratio (pot experiment and both  
225 experiments combined, Table 4). Also, fertilization effects on the foliar nutrient concentrations  
226 differed distinctly from the effects on the soil. The most prominent and strongly significant  
227 effect was a decreased Mg concentration, and a respective increase in N:Mg (Table 4 and  
228 Supplementary Table). A similar effect was observed for the micro-nutrients B and Zn  
229 (Supplementary Table). In addition, fertilization led to a small but significant increase in N in  
230 the field experiment, to a small but significant decrease in Ca in the pot experiment, and to  
231 related significant effects on the N:P ratio in the field experiment and on the N:Ca ratio in the  
232 pot experiment (Tables 1, 2, and 4). All the latter effects were also significant when considering  
233 both experiments combined by LME modelling (Table 4).

234 In the case of the pot experiment, nutrient contents in the stem (concentrations x biomass)  
235 provided a different picture (Supplementary Table). Here, fertilization significantly increased  
236 the uptake of all measured nutrients ( $P < 0.001$  for N, P, K, S, Ca;  $P = 0.002$  for Mg), whereas  
237 CaCO<sub>3</sub> addition tended to increase Ca uptake only when combined with fertilization.

238

### 239 *3.3 Plant growth*

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

248 When considering both experiments combined by LME modelling, the fertilization effects  
249 were highly significant for diameter and height, while the significance level was lower for  
250 biomass (Table 5). CaCO<sub>3</sub> addition had only weak increasing effects on diameter in the field  
251 experiment, and on biomass, when considering both experiments combined (Table 5).

252

## 253 **4. Discussion**

### 254 *4.1 Growth response to calcium carbonate and NPKS fertilization*

255 Taking the results on the growth response of poplar from our two experiments together,  
256 indicates that fertilization effects on growth were stronger when soil pH and exchangeable Ca  
257 values were above a certain level, thus confirming our hypothesis. Although the main effect of  
258 the fertilizer addition on soil was an increase in available P and K, indications from foliar nu-  
259 trient analyses point rather to N as a limiting nutrient, as is argued in the following section. The

260 overall stronger effect of fertilization in the pot than the field experiment can be explained by  
261 the higher starting pH and CaCO<sub>3</sub> containing irrigation solution in the pot experiment. Thus, in  
262 this experiment soil pH was at a sufficiently high level to make fertilization effective even  
263 without CaCO<sub>3</sub> addition. As is further elaborated in the following section, in this context not  
264 only soil pH is important but also the plant availability of Ca, thus suggesting an additional  
265 limiting effect of Ca on growth. Thus, our results not only support earlier findings on the effect  
266 of soil pH on the growth of poplar (Coudoun et al., 2006; Hjelm and Rytter, 2016), but also  
267 emphasize the importance of Ca in cell wall development and thus wood formation in poplar  
268 (Lautner et al., 2007; Baribault et al., 2012).

269

#### 270 *4.2 Effects of soil amendments on soil pH, available soil nutrients and plant nutritional status*

271 In poplar plantations on acid soils, calcium carbonate is often used to increase soil pH  
272 (Böhlenius et al. 2020) and preferred to other forms of calcium containing materials such as  
273 e.g. gypsum, which does not increase soil pH. In our study, calcium carbonate increased soil  
274 pH in both experiments, irrespective of fertilization. The generally higher pH in the pot than  
275 the field experiment can be explained on one hand by the higher pH of the soil at the beginning  
276 of the experiment and by the carbonate containing irrigation water used in the pot experiment  
277 (as compared to water provided by rainfalls only in the field experiment with a pH below 7).  
278 Considering the optimum pH values for growth of poplar found to be between 6.0 and 6.5  
279 (Dickmann et al., 2001), the consequence of the differences in starting and irrigation conditions  
280 was that in the pot experiment, already the lowest CaCO<sub>3</sub> dosage was sufficient to reach this  
281 pH level, whereas higher dosages were needed in the field experiment. Considering the overall  
282 consistent linear relationship between pH and exchangeable Ca in the two experiments (Fig. 2),  
283 the apparent failure of the highest dosage in the field experiment to increase the pH even higher  
284 is likely due to soil heterogeneity.

285 Overall, the effects of the soil amendments on available nutrient concentrations in the soil,  
286 i.e. an increase in Ca by CaCO<sub>3</sub> additions, and increase in K and P by fertilization, were not  
287 reflected by a respective increase in foliar concentrations. However, the results on nutrient  
288 contents in the stem in the pot experiment indicate that uptake of all nutrients was increased by  
289 fertilization. The decreased foliar concentrations of Mg and the micronutrients B and Zn in the  
290 fertilized treatments, nutrients added by neither amendment, indicate limited availability of  
291 these elements in the soil.

292 The foliar element concentrations and element ratios also provide some clues with respect to  
293 limiting nutrients. The positive effect of fertilization on the foliar N concentration and N:P ratio,  
294 while there was no effect on the mineral N concentrations in the soil, points to N limitation of  
295 plant growth. Under such conditions, plant uptake – and possibly microbial immobilization –  
296 would keep concentrations of available N in the soil low, irrespective of N addition. According  
297 to our hypothesis, the positive effect of CaCO<sub>3</sub> addition on plant nutrition could on one hand  
298 be attributed to an increase in soil pH above a value where sorption of anionic nutrients, in  
299 particular phosphate, becomes negligible with a positive effect on P availability, or to an  
300 increase in available Ca. Considering the close relationship between soil pH and exchangeable  
301 Ca, when combining the results of our experiments, the soil data do not allow us to distinguish  
302 between the two potential effects. However, the weak decreasing trend in foliar P  
303 concentrations with increasing CaCO<sub>3</sub> additions in both experiments may point to an additional  
304 role of Ca phosphate in controlling P availability, while the weakening effect on phosphate  
305 sorption should become larger with increasing CaCO<sub>3</sub> addition and thus pH. This further  
306 suggests that a positive effect of CaCO<sub>3</sub> addition on plant growth may at least partly be  
307 attributed to relieving Ca limitation.

308

309 **Conclusion**

310 This study evaluated the effects of the simultaneous application of calcium carbonate with  
311 NPKS fertilization on growth of poplar on acid soils based on two twin experiments in open  
312 field and in pots. Although the use of two different clones may explain some of the differences  
313 in plant growth and foliar nutrient concentrations between the two experiments, taken together  
314 the results indicate that fertilization effects on growth were stronger when soil pH and, related  
315 to this, exchangeable Ca were above a certain threshold. Thus, the results of this study on one  
316 hand emphasize the importance of adapting the level of CaCO<sub>3</sub> to the given soil and irrigation  
317 conditions, and on the other hand ask for further studies addressing the relative importance of  
318 elevated pH and improved Ca nutrition.

319

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327

328 **References**

- 329 Baribault, T.W., Kobe, R.K., Finley, A.O., 2012. Tropical tree growth is correlated with soil  
330 phosphorus, potassium, and calcium, though not for legumes. *Ecol. Monogr.*  
331 <https://doi.org/10.1890/11-1013.1>
- 332 Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models  
333 using lme4. *J. Stat. Softw.* 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- 334 Beaudoin, N., Saad, J.K., Van Laethem, C., Machet, J.M., Maucorps, J., Mary, B., 2005.  
335 Nitrate leaching in intensive agriculture in Northern France: Effect of farming practices,  
336 soils and crop rotations. *Agric. Ecosyst. Environ.*  
337 <https://doi.org/10.1016/j.agee.2005.06.006>
- 338 Bergante, S., Facciotto, G., Marchi, M., 2020. Growth dynamics of “Imola” poplar clone  
339 (*Populus × canadensis* Mönch) under different cultivation inputs. *Ann. Silv. Res.* 44,  
340 71–79. <https://doi.org/10.12899/asr-1934>
- 341 Böhlenius, H., Nilsson, U., Salk, C., 2020. Liming increases early growth of poplars on forest  
342 sites with low soil pH. *Biomass and Bioenergy* 138.  
343 <https://doi.org/10.1016/j.biombioe.2020.105572>
- 344 Bünemann, E.K., Keller, B., Hoop, D., Jud, K., Boivin, P., Frossard, E., 2013. Increased  
345 availability of phosphorus after drying and rewetting of a grassland soil: Processes and  
346 plant use. *Plant Soil* 370, 511–526. <https://doi.org/10.1007/s11104-013-1651-y>
- 347 Coleman, M., Tolsted, D., Nichols, T., Johnson, W.D., Wene, E.G., Houghtaling, T., 2006.  
348 Post-establishment fertilization of Minnesota hybrid poplar plantations. *Biomass and*  
349 *Bioenergy.* <https://doi.org/10.1016/j.biombioe.2006.01.001>
- 350 De Vries, W., Posch, M., 2011. Modelling the impact of nitrogen deposition, climate change  
351 and nutrient limitations on tree carbon sequestration in Europe for the period 1900-2050.  
352 *Environ. Pollut.* <https://doi.org/10.1016/j.envpol.2010.11.023>
- 353 Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R.E., Khan, Z., Ko, A.W.K.,

354 Kim, S.H., DeLuca, T.H., 2016. Variable nitrogen fixation in wild *Populus*. PLoS One.  
355 <https://doi.org/10.1371/journal.pone.0155979>

356 FAO, 2016. Poplars and other fast-growing trees - renewable resources for future green  
357 economies. Synth. Ctry. Prog. Reports. 25th Sess. Int. Poplar Comm. Berlin, Fed. Repub.  
358 Ger.

359 Fernández, F., Hoefl, R., 2009. Managing soil pH and crop nutrients. Illinois Agron. Handb.  
360 91–112.

361 Foster, N.W., Bhatti, J.S., 2006. Forest ecosystems: nutrient cycling. *Encycl. Soil Sci.*

362 Georgiadis, P., Taeroe, A., Stupak, I., Kepfer-Rojas, S., Zhang, W., Pinheiro Bastos, R.,  
363 Raulund-Rasmussen, K., 2017. Fertilization effects on biomass production, nutrient  
364 leaching and budgets in four stand development stages of short rotation forest poplar.  
365 *For. Ecol. Manage.* <https://doi.org/10.1016/j.foreco.2017.04.020>

366 Grover, Z.S., Cook, R.L., Zapata, M., Byron Urrego, J., Albaugh, T.J., Zelaya, A., Ozyhar, T.,  
367 Rubilar, R., Carter, D.R., Campoe, O.C., 2021. *Eucalyptus grandis* Response to Calcium  
368 Fertilization in Colombia. *For. Sci.* 67, 701–710.  
369 <https://doi.org/10.1093/FORSCI/FXAB042>

370 Hacke, U.G., Plavcová, L., Almeida-Rodriguez, A., King-Jones, S., Zhou, W., Cooke, J.E.K.,  
371 2010. Influence of nitrogen fertilization on xylem traits and aquaporin expression in  
372 stems of hybrid poplar. *Tree Physiol.* <https://doi.org/10.1093/treephys/tpq058>

373 Han, S.H., An, J.Y., Hwang, J., Kim, S. Bin, Park, B.B., 2016. The effects of organic manure  
374 and chemical fertilizer on the growth and nutrient concentrations of yellow poplar  
375 (*Liriodendron tulipifera* Lin.) in a nursery system. *Forest Sci. Technol.*  
376 <https://doi.org/10.1080/21580103.2015.1135827>

377 Hjelm, K., Rytter, L., 2016. The influence of soil conditions, with focus on soil acidity, on the  
378 establishment of poplar (*Populus* spp.). *New For.* 47, 731–750.  
379 <https://doi.org/10.1007/s11056-016-9541-9>

380 Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S.,  
381 Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils,  
382 crops and biodiversity in the UK: A review. *Sci. Total Environ.*  
383 <https://doi.org/10.1016/j.scitotenv.2017.08.020>

384 Jug, A., Hofmann-Schielle, C., Makeschin, F., Rehfues, K.E., 1999. Short-rotation  
385 plantations of balsam poplars, aspen and willows on former arable land in the Federal  
386 Republic of Germany. II. Nutritional status and bioelement export by harvested shoot  
387 axes. *For. Ecol. Manage.* [https://doi.org/10.1016/S0378-1127\(98\)00557-X](https://doi.org/10.1016/S0378-1127(98)00557-X)

388 Lautner, S., Ehling, B., Windeisen, E., Rennenberg, H., Matyssek, R., Fromm, J., 2007.  
389 Calcium nutrition has a significant influence on wood formation in poplar. *New Phytol.*  
390 173, 743–752. <https://doi.org/10.1111/j.1469-8137.2007.01972.x>

391 R Development Core Team, 2020. R: A language and environment for statistical computing.  
392 R Found. Stat. Comput. <https://doi.org/10.1017/CBO9781107415324.004>

393 Reid, C., Watmough, S.A., 2014. Evaluating the effects of liming and wood-ash treatment on  
394 forest ecosystems through systematic meta-analysis. *Can. J. For. Res.* 44, 867–885.  
395 <https://doi.org/10.1139/cjfr-2013-0488>

396 Rennenberg, H., Wildhagen, H., Ehling, B., 2010. Nitrogen nutrition of poplar trees. *Plant*  
397 *Biol.* 12, 275–291. <https://doi.org/10.1111/j.1438-8677.2009.00309.x>

398 Schmidt, M., Veldkamp, E., Corre, M.D., 2015. Tree species diversity effects on productivity,  
399 soil nutrient availability and nutrient response efficiency in a temperate deciduous forest.  
400 *For. Ecol. Manage.* 338, 114–123. <https://doi.org/10.1016/j.foreco.2014.11.021>

401 Winans, K.S., Tardif, A.S., Lteif, A.E., Whalen, J.K., 2015. Carbon sequestration potential  
402 and cost-benefit analysis of hybrid poplar, grain corn and hay cultivation in southern  
403 Quebec, Canada. *Agrofor. Syst.* <https://doi.org/10.1007/s10457-014-9776-4>

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**Table 1.** pH in topsoil, nutrient concentrations in topsoil (exchangeable Ca, K, SO<sub>4</sub>; mineral N, i.e. the sum of NH<sub>4</sub>-N and NO<sub>3</sub>-N; resin P) and green leaves (total element concentrations) of the field experiment; shown are Mean  $\pm$  SD for fertilized and not fertilized plots with a given CaCO<sub>3</sub> addition (A), and for plots without or with fertilization, irrespective of CaCO<sub>3</sub> addition (B). Superscript letters (a, ab, b) refer to a post-hoc test we performed on the data.

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

**Table 2.** pH in topsoil, nutrient concentrations in topsoil (exchangeable Ca, K, SO<sub>4</sub>; mineral N, i.e. the sum of NH<sub>4</sub>-N and NO<sub>3</sub>-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<sub>3</sub> addition (A), and for pots without or with fertilization, irrespective of CaCO<sub>3</sub> addition (B). Superscript letters (a, ab, b) refer to a post-hoc test we performed on the data.

	Topsoil concentrations [mg kg <sup>-1</sup> ]						Total foliar concentrations [g kg <sup>-1</sup> ]				
	pH topsoil	Ca_ex	N_min	P_resin	K_ex	SO4_ex_S	Ca	N	P	K	S
<b>A</b>											
C0	5.57 <sup>c</sup> ± 0.22	880 <sup>b</sup> ± 205	1.2 <sup>a</sup> ± 0.7	5.6 <sup>a</sup> ± 6.8	69 <sup>a</sup> ± 14	2.7 <sup>a</sup> ± 1.2	13.4 <sup>a</sup> ± 2.1	21.9 <sup>a</sup> ± 5.2	1.74 <sup>a</sup> ± 0.18	9.8 <sup>a</sup> ± 2.7	3.51 <sup>a</sup> ± 1.12
C500	5.98 <sup>bc</sup> ± 0.44	1169 <sup>b</sup> ± 228	1.2 <sup>a</sup> ± 0.4	6.6 <sup>a</sup> ± 7.2	73 <sup>a</sup> ± 14	2.1 <sup>a</sup> ± 0.6	16.0 <sup>a</sup> ± 3.7	16.9 <sup>a</sup> ± 0.8	1.60 <sup>a</sup> ± 0.17	8.6 <sup>a</sup> ± 2.7	3.37 <sup>a</sup> ± 0.81
C1000	6.24 <sup>b</sup> ± 0.45	1350 <sup>b</sup> ± 502	0.9 <sup>a</sup> ± 0.7	6.8 <sup>a</sup> ± 6.8	72 <sup>a</sup> ± 11	2.8 <sup>a</sup> ± 1.3	15.6 <sup>a</sup> ± 2.9	17.8 <sup>a</sup> ± 3.8	1.60 <sup>a</sup> ± 0.23	8.6 <sup>a</sup> ± 2.1	3.30 <sup>a</sup> ± 0.52
C1500	6.40 <sup>b</sup> ± 0.37	1332 <sup>b</sup> ± 238	1.5 <sup>a</sup> ± 1.2	5.3 <sup>a</sup> ± 5.1	73 <sup>a</sup> ± 15	2.6 <sup>a</sup> ± 1.0	14.3 <sup>a</sup> ± 1.1	17.5 <sup>a</sup> ± 3.1	1.54 <sup>a</sup> ± 0.17	8.0 <sup>a</sup> ± 1.1	3.26 <sup>a</sup> ± 0.50
C2000	6.71 <sup>a</sup> ± 0.25	2073 <sup>a</sup> ± 709	1.2 <sup>a</sup> ± 0.5	7.0 <sup>a</sup> ± 7.6	73 <sup>a</sup> ± 12	3.0 <sup>a</sup> ± 1.3	13.2 <sup>a</sup> ± 2.1	17.3 <sup>a</sup> ± 2.2	1.52 <sup>a</sup> ± 0.21	8.0 <sup>a</sup> ± 1.6	3.23 <sup>a</sup> ± 0.72
<b>B</b>											
without fertilization	6.18 <sup>a</sup> ± 0.48	1317 <sup>a</sup> ± 653	1.3 <sup>a</sup> ± 0.8	1.9 <sup>b</sup> ± 0.9	67 <sup>b</sup> ± 11	2.3 <sup>a</sup> ± 1.0	15.8 <sup>a</sup> ± 2.4	17.6 <sup>a</sup> ± 3.1	1.61 <sup>a</sup> ± 0.18	8.0 <sup>a</sup> ± 1.6	3.57 <sup>a</sup> ± 0.48
with fertilization	6.18 <sup>a</sup> ± 0.56	1404 <sup>a</sup> ± 480	1.1 <sup>a</sup> ± 0.6	10.6 <sup>a</sup> ± 6.7	77 <sup>a</sup> ± 13	3.0 <sup>a</sup> ± 1.1	13.2 <sup>b</sup> ± 2.2	19.0 <sup>a</sup> ± 4.1	1.59 <sup>a</sup> ± 0.22	9.3 <sup>a</sup> ± 2.4	3.10 <sup>a</sup> ± 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, SO<sub>4</sub>, Mg; mineral N, i.e. the sum of NH<sub>4</sub>-N and NO<sub>3</sub>-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 <sub>3</sub>	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 <sub>4</sub> _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:  $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05$

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:  $0 \leq *** < 0.001 \leq ** < 0.01 \leq * < 0.05$ -

Parameter	Fixed-effect	MIXED EFFECTS MODEL						ANOVA ON	ANOVA ON
		Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	FIELD DATA	POT DATA
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  $\text{CaCO}_3$  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.

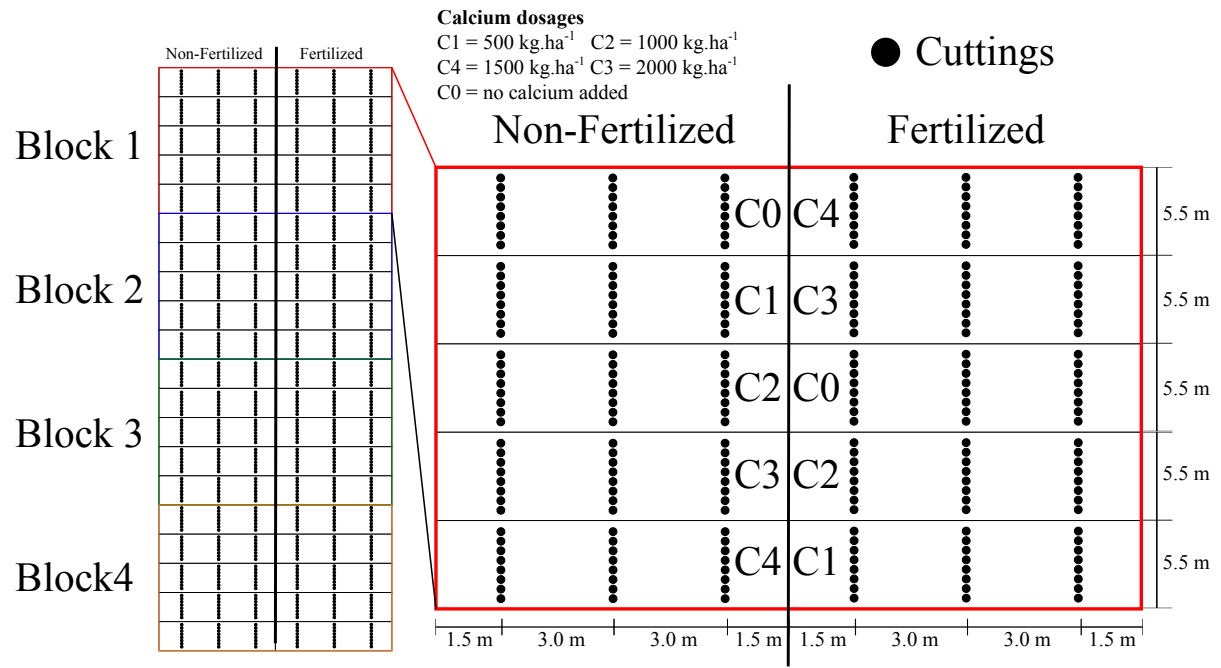


Fig. 1

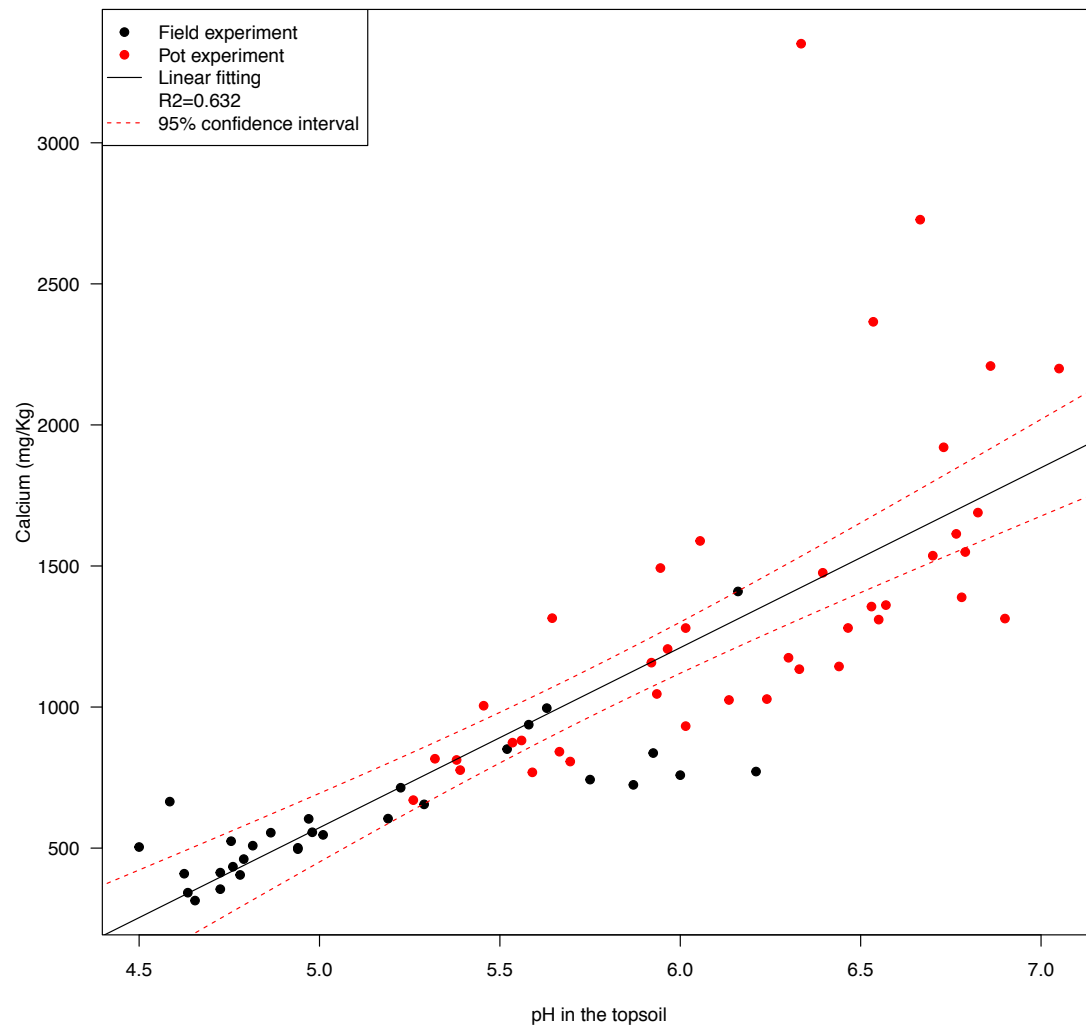


Fig. 2



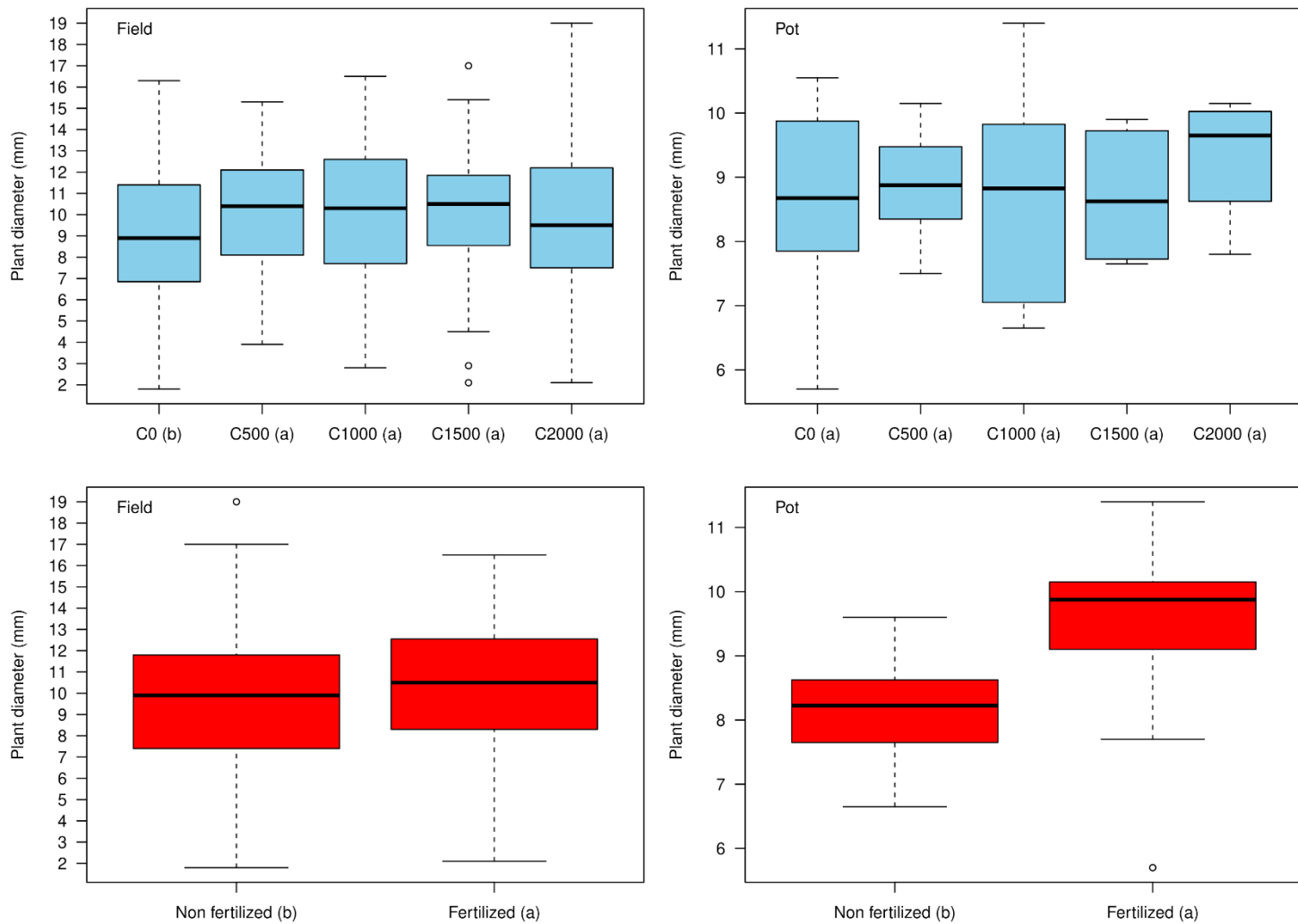


Fig 3

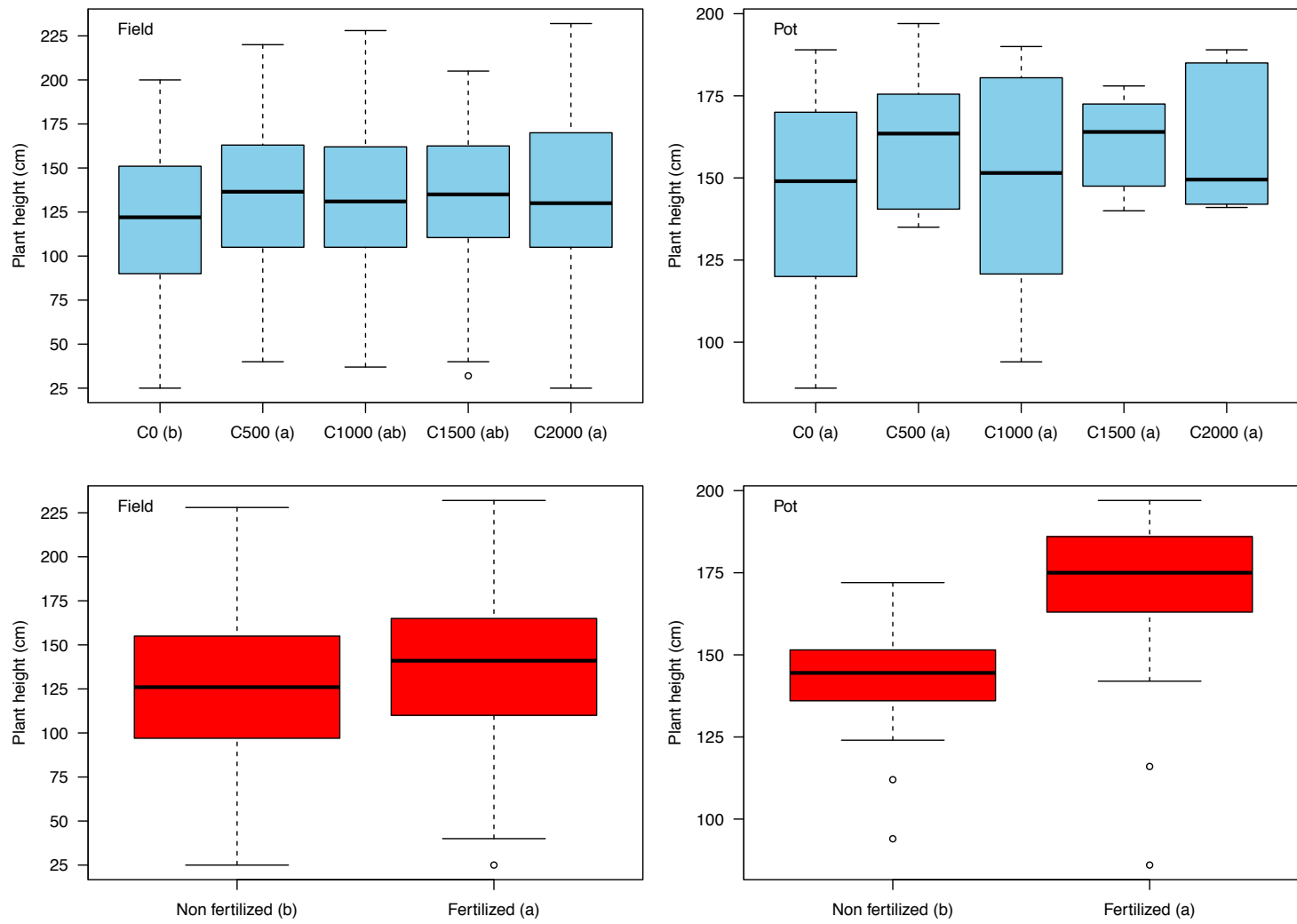


Fig. 4

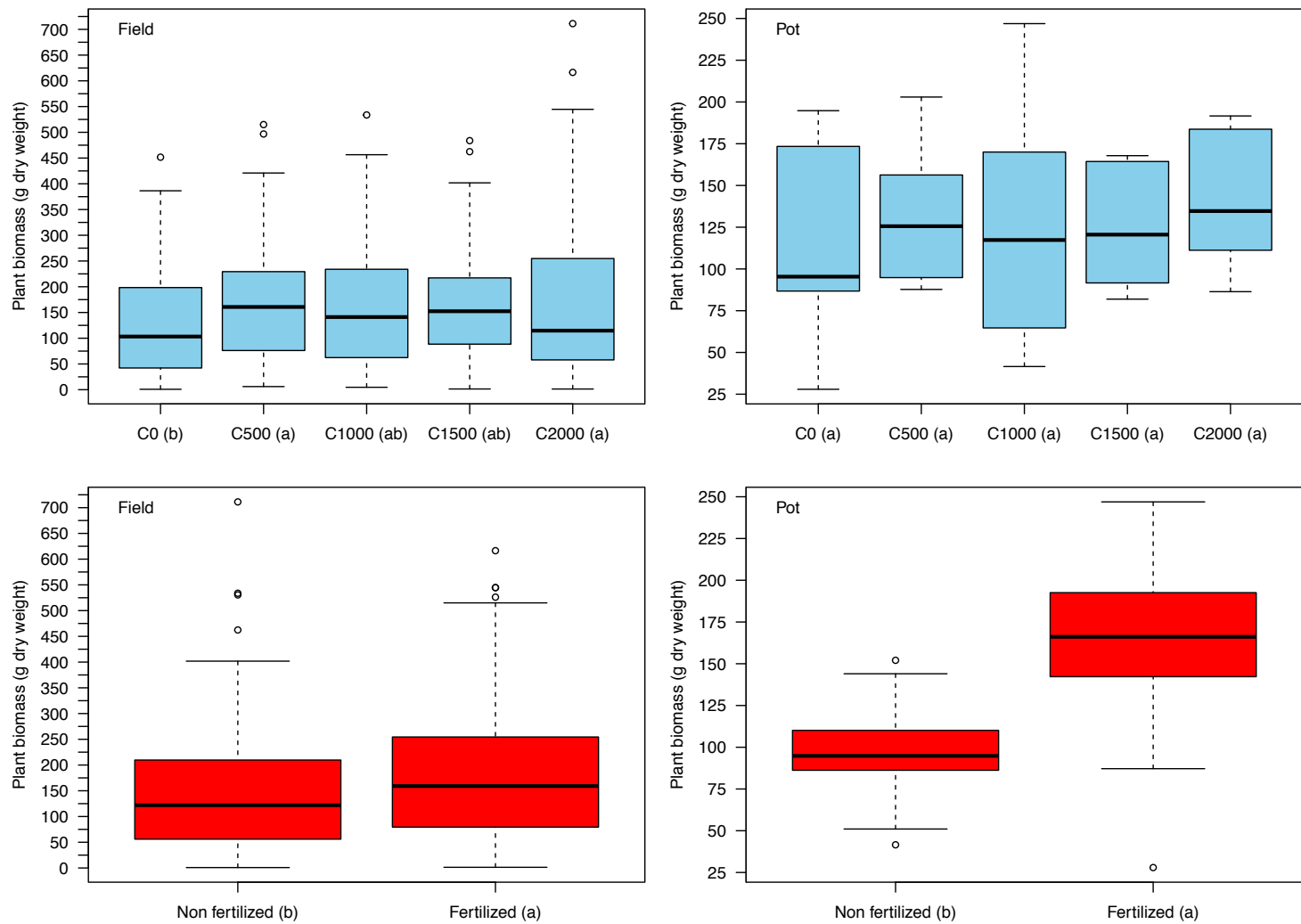
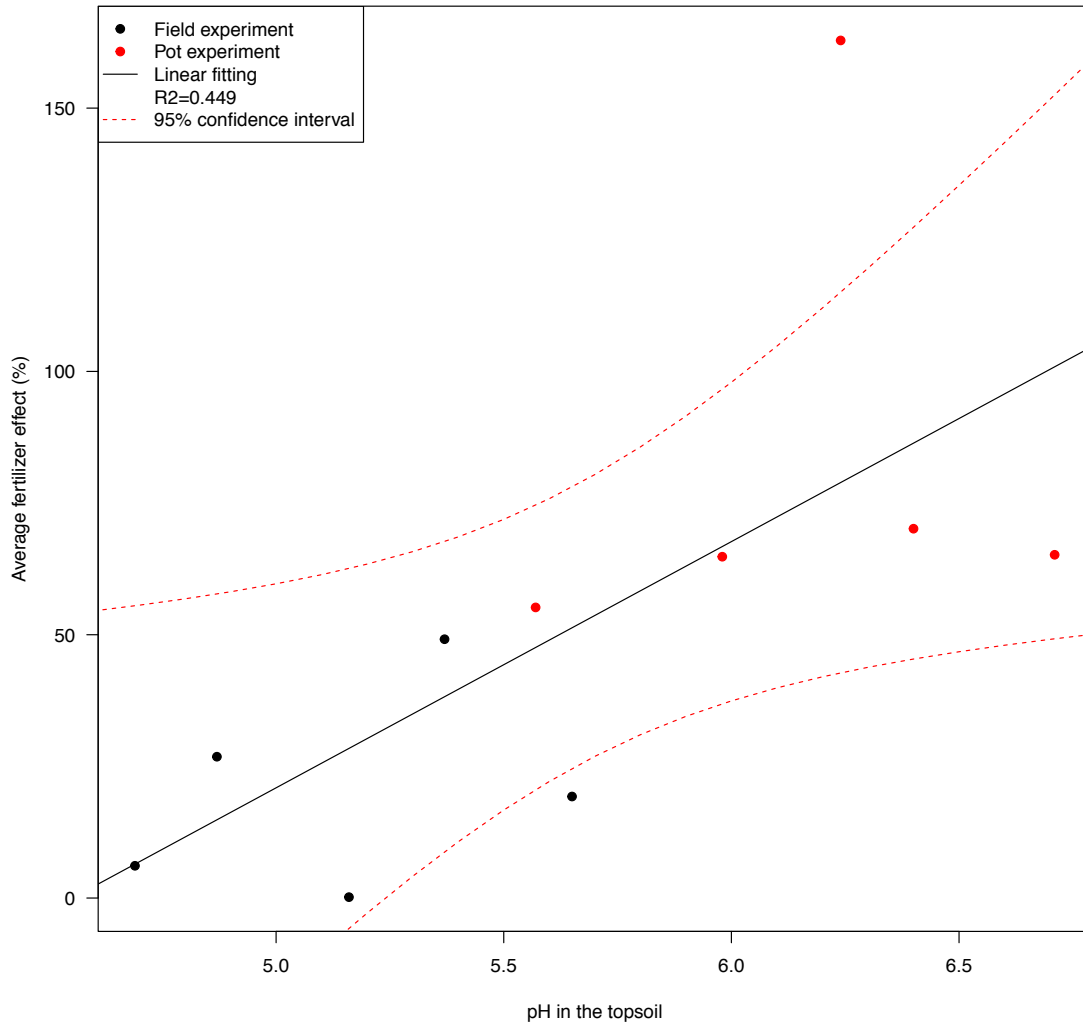


Fig. 5



1

2 **Fig. 6**

3