Nutritional status and genetic variation in the response to nutrient availability in *Pinus pinaster*. A multisite field study in NW Spain

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Abstract

The low nutrient availability of the acidic and sandy soils of Galicia (NW Spain) is probably the main environmental factor limiting forest primary productivity in the area. These particular edaphic conditions could have imposed selective pressures on maritime pine populations leading to specific local adaptations.

We first assessed the nutritional status of 22 young contemporary *P. pinaster* plantations in NW Spain, and then analysed the response to fertilization in three family × fertilization trials, and how this response varied across sites and genotypes.

Growth of *P. pinaster* in NW Spain appeared to be largely limited by nutrient availability, where most of the plantations showed severe nutrient deficiencies, especially in P and Mg. According to these deficiencies, a strong positive response to nutrient additions was observed in the three trials, with height increments of up to 30% compared with the unfertilized control. However, the response to fertilizers was very variable from site to site, and in some cases did not agree with the foliar nutritional diagnosis. The response to fertilization was also significantly affected by pine genotype, suggesting that the plastic response to nutrient additions within each environment was under genetic control. However, the family response to nutrient availability was not consistent across sites, and no significant differences among families were observed for the RDPI plasticity index –a single index that summarizes the phenotypic change in multiple environments– when analysed across environments.

The strong environmental component modulating phenotypic responses to fertilization could impose an important obstacle to evolve specific adaptations to the local edaphic conditions, as well as to artificially select genotypes adapted to different environments and silviculture regimes.

Keywords: Genetic variation, Phenotypic plasticity, Genotype × environment interaction, Fertilization × genotype interaction, Nutrient deficiencies, Plasticity index.

Introduction

Within its natural range of distribution, maritime pine (*Pinus pinaster* Ait.) populations show high geographical structuration of intraespecific genetic variation (Gonzalez Martinez *et al.*, 2004; Bucci *et al.*, 2007). The extremely variable ecological conditions where this species grows and the isolation of populations have led to several adaptations to local environmental conditions, such as specific parent materials, drought regimes, or fire frequency (e.g. Alia *et al.*, 1997; Tapias *et al.*, 2004; Correia *et al.*, 2008). Within population genetic variation is also high, favouring the ability to evolve in response to local selective pressures (Gonzalez Martinez *et al.*, 2005). In addition, *P. pinaster* is recognized to show a high phenotypic plasticity, being able to modulate the phenotype expression according to the particular environmental conditions where it grows (Chambel *et al.*, 2007).

Forest soils in Northwest Spain typically show low nutrient availability and large spatial heterogeneity in chemical soil properties, both at small and large scales (Paz-Gonzalez *et al.*, 2000; Gallardo and Covelo, 2005). They are acidic (as results from humid climate, subtractive systems, and acid parent materials) and they have high organic matter content as a consequence of the slow mineralization rates due to Aluminum binding (Calvo and Díaz-Fierros, 1982). The high precipitation rates and intensities in the area and the sandy textures of the soils facilitate infiltration and cation leaching, which, together with the low pH, results in low nutrient availabilities. However, nutrient richness largely varies across the diverse geological materials found in the region (Macías *et al.*, 1982), and diverse ecological processes at the individual or ecosystem scale also generate small scale spatial heterogeneity in chemical soil properties (Gallardo and Covelo, 2005).

Due to high mean annual temperatures and rainfall, and favourable light availabilities, the net primary forest productivity in this region is relatively high. The poor quality of the soils, however, is commonly displayed in important nutrient deficiencies in

forest stands that limit their potential growth (e.g. Merino *et al.*, 2003; Zas and Serrada, 2003). Phosphorus, potassium and magnesium are the main factors limiting tree growth in this region. Soil nitrogen content is usually sufficient for forest production, and may even be excessive, unbalancing the nutrition with other macronutrients (Zas and Serrada, 2003). Reflecting these nutrient deficiencies, large responses of forest plantations to nutrient additions have been widely reported in the area, and forest fertilization has been strongly recommended (Solla-Gullón *et al.*, 2004; Solla-Gullón *et al.*, 2006; Zas *et al.*, 2006a).

Almost all work analyzing the nutritional status and fertilization needs of forest plantations in NW Spain has been carried out on fast growing exotic species such as *Pinus radiata* (Sánchez-Rodríguez *et al.*, 2002; Zas and Serrada, 2003), *Pseudotsuga menziesii* (Zas, 2003; Solla-Gullón *et al.*, 2006) or *Eucalyptus globulus* (Merino *et al.*, 2003). The nutritional disorders commonly observed in these studies have been attributed to the high specific nutrient demand of these highly productive species. However, no information on nutritional aspects is available for the maritime pine (*Pinus pinaster* Ait.), a fast growing native colonizer, widely spread in SW Europe.

Maritime pine populations from NW Spain are included within the Atlantic group, which also includes the well known Landas French provenances and Leiría Portuguese provenances, broadly used for forestry purposes. However, several studies using molecular markers (Bucci *et al.*, 2007), or analyzing quantitative variation in provenance trials (Molina, 1965; Alia *et al.*, 1997) have revealed the singularity of the Galician maritime pine origin within the Iberian Peninsula context, and even within the Atlantic group. The particular edaphic conditions in this area may have contributed to the differentiation of these populations through local adaptations to low and heterogeneous nutrient availability. In concordance with this hypothesis, it is axiomatic among local foresters that *P. pinaster* is much less nutrient demanding and responds much less to fertilization than other fast growing species (Bará, 1990).

The aims of the present study were i) to check if *P. pinaster* plantations in NW Spain show nutritional disorders as has been demonstrated for other fast growing tree species in the region, ii) to determine the response to fertilization with different nutrients of *P. pinaster* and how this response varied across different test sites, i.e. how the response to nutrient availability is environmentally modulated, and iii) to quantify the genetic control of the phenotypic responses to nutrient availability in the north west maritime pine population.

Material and Methods

Experimental approach

In order to answer to the first question, twenty-two *P. pinaster* reforestations, planted throughout Galicia (NW Spain) in 1996 and representative of the maritime pine stands in the region, were selected and sampled for nutritional diagnosis (Figure 1).

In addition, three family × fertilization trials were established in 2003 (sites B and C) and in 2004 (site A) to analyze the response of *P. pinaster* to establishment fertilization and to quantify the variation in the response to nutrient availability in the NW Coastal *P. pinaster* population (Figure 1).

Climate throughout the area is temperate humid Atlantic, with high annual precipitation and relatively short summer drought (Table 1). The trial at site A has a slight Mediterranean and Continental influences, with lower annual and summer precipitation and higher temperature fluctuation than sites B and C.

Plant material

All the *P. pinaster* reforestation stands were planted in 1996 with genetic material from the Region of Provenance ‘1a Coastal Galicia’ (Alía *et al.*, 1996).

The plant material of the family × fertilization trials consisted of open-pollinated families from 28 maternal plus trees selected for superior growth, stem form and branch characteristics in mature plantations or natural stands of *P. pinaster* in Galicia. All seeds were collected in a clonal seed orchard (Sergude, 42.82ºN, 8.45ºW) in the same year. We also included three unimproved commercial *P. pinaster* seed sources as controls, two from Galicia and one from France (Landas origin). The experimental units were completed to 32 plants with one *P. radiata* extra seed source, not included in the analysis.

Experimental layout

Reforestation stands were sampled when they were around two years old. Within each site, one rectangular sample plot of 50 forested plants was established for each hectare planted. Sample plot size was variable depending on the planting density, with a minimum area of 300 m^2.

The experimental layout in the three family × fertilization trials was a split-plot design replicated in ten blocks, with nine fertilization treatments acting as the main factor and the genetic entries as the split factor. In total, we planted 2880 seedlings in each trial, corresponding to 10 blocks × 9 fertilization treatments × 32 genetic entries (28 families of half-sibs + 3 control seed lots + 1 *P. radiata*). Spacing was 3 × 2 m. Fertilization treatments were an unfertilized control and eight treatments built upon combinations of four commercial fertilizers (Table 2). Plants were fertilized by hand just after planting.

Sampling and field assessments

Total height and root-collar diameter were measured in all living plants in the sample plots of the 22 reforestation stands at age 2. In the family × fertilization trials, total height in cm was measured in all living plants yearly during the first five years after planting.

For foliar nutritional diagnosis of all stands (reforestations and family × fertilization trials), current season needle samples were collected from three branch-ends per tree in the upper third of the crowns of the trees following Ballard et al. (1986). In the 22 reforestation stands, two composite samples (from a minimum of 10 trees each) were collected in each sampling plot at the end of the second growing season.

Foliar nutrient diagnosis in the three family × fertilization trials was carried out on the unfertilized 'control' plants five years after planting. In these trials a more extensive sampling was carried out, with the collection of five composite samples in each trial following the same procedure as above. Samples were placed in plastic bags and transported in ice-cooled containers to the lab. Needle samples were oven-dried at 65 ºC to constant weight, finely grounded and preserved for chemical analysis.

Composite soil samples were also taken in all stands for chemical analysis. In the reforestation stands, five soil samples (0–20 cm depth, 8 cm diameter soil corers) were taken from the four corners and centre of each sampling plot, mixed, and homogenized to form one composite sample per plot. In the progeny × fertilization trials, three composite soil samples (composed of five samples each as above) were taken per trial. Soil samples were sieved through a 2 mm screen, oven-dried at 60 ºC and stored until chemical analysis.

Plant and Soil Analyses

The foliar contents of total P, K, Ca and Mg were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Perking-Elmer Optima 4300DV (Massachusetts, USA) after microwave assisted digestion of foliar samples. Total N concentrations were determined by dry combustion using a LECO CN-2000 elemental analyzer (LECO Corporation, St. Joseph, MI). The foliar nutrient critical levels reported by Bonneau (1995) for N, P, K, and Ca nutrition in *P. pinaster*, and by Will (1985) for Mg nutrition in *P. radiata* were used for nutritional diagnosis of all stands.

For soil analyses, total N and total organic C were determined using a LECO CN-2000 elemental analyzer (LECO Corporation, St. Joseph, MI); total K, Ca and Mg were determined by ICP-OES using a Perking-Elmer Optima 4300DV (Massachusetts, USA) after microwave assisted wet digestion as above; and available P was evaluated by the Bray-II method (Bray and Kurtz, 1945) (UV-VIS Beckman). The particle-size distribution in soil samples was determined following the pipette method (Gee and Bauder, 1996), and the pH was measured in deionized H_2O (1 : 2.5 d.w. : vol).

Chemical analyses were performed in the central laboratory facilities (C.A.C.T.I.) at Universidade de Vigo (http://webs.uvigo.es/cactiweb/index.htm).

Adjusting spatial autocorrelation

Growth traits assessed in field trials frequently show non-random spatial structures that may affect the efficiency of standard statistical analyses (e.g. Fu *et al.*, 1999; Dutkowski *et al.*, 2006). When spatial heterogeneity is present, near neighbours are more similar than plants further away, i.e., data are autocorrelated, and the requirement of data independence in standard parametric statistics is violated (Legendre, 1993). Although block designs improve the statistical efficiency by controlling some of the spatial variation, there are many situations where block designs are not able to account for all the spatial heterogeneity. In such cases, spatial analysis techniques become essential to correctly analyze spatial autocorrelated data (Dutkowski *et al.*, 2006; Zas, 2006).

We determined the spatial position of each tree using a total station (Pentax R-315) and then we prospected the spatial structure of the dependent variable in each site by constructing the empiric semivariogram for the residuals adjusted for main effects (fertilization, family, and family × fertilization) with the SAS VARIOGRAM procedure (SAS-Institute, 1999). Those variables that were spatially dependent were corrected using the Iterative Spatial Analysis (ISA) method (Zas, 2006). Briefly, the spatial variation of the

dependent variable was modeled by the kriging method using a theoretical model fitted to the observed semivariogram and the KRIG2D procedure of SAS. We then adjusted the original variable for its spatial autocorrelation, subtracting the kriging estimate in each position. The new corrected variable was then reanalysed and a new estimate of the main effects was obtained, and used to generate new residuals. The process was repeated iteratively, until convergence of the estimates of main effects (usually 5 steps are enough). A detailed description of the method can be consulted in Zas (2006). The spatial adjustments were carried out for each site (A, B, and C) and age (1 to 5) independently.

Statistical analyses

Spatially adjusted height data at each age in the three family × fertilization trials were analysed by means of a split-split model, in which the site (S) was assigned to the main plot, the fertilization treatments (F) to the subplot, and the genotypes (open-pollinated families, G) to the sub-subplot. The data were analysed with a mixed model using the SAS MIXED procedure (Littell *et al.*, 2006).

To test for significant effects of including a specific nutrient in the fertilization treatment, specific comparisons between those treatments that only differ in the inclusion of that nutrient were analysed using the CONTRAST statement of the MIXED procedure (Littell *et al.*, 2006). The effect of including N was tested contrasting treatments (F_1, F_3, F_5, F_7) with treatments (F_2, F_4, F_6, F_8) (see Table 2 for treatment description). The effects of adding PCa, K, and Mg were tested with the following specific contrasts: (F_1, F_2) versus (F_3, F_6) for P, (F_1, F_2) versus (F_4, F_7) for K, and (F_1, F_2) versus (F_5, F_8) for Mg.

Variation in the response to nutrient availability

The Simplified Relative Distances Plasticity Index ($RDPI_s$) proposed by Valladares et al. (2006) was used to estimate, within each site and age, the response of each *P. pinaster*

family to variation in nutrient availability. This index is particularly suited for quantifying the phenotypic change of a given genetic entry along more than 3 environments that do not follow a specific environmental gradient. The index was calculated based on phenotypic distances between the least square means for each family-treatment combination derived from the mixed models. For each age and site, the $RDPI_s$ index for genotype *i* was calculated as:

$$RDPI_s(i) = \frac{\sum_{j \neq k} \left| LSM_{ij} - LSM_{ik} \right| / (LSM_{ij} + LSM_{ik})}{n}$$

where LSM_{ij} and LSM_{ik} are the least squares for height growth of genotype *i* in treatments *j* and *k*, respectively (being *j* and *k* always different fertilization treatments), and *n* is the total number of distances, i.e. the number of possible pairs of different treatments. In our case, with 9 fertilization treatments, $n = 36$.

The $RDPI_s$ indices were calculated for each site independently, resulting in three estimations of the plastic responses for each family and age. For each age, a two way ANOVA was applied to the $RDPI_s$ data to test for the environmental (site) and genetic (family) control of the response to variation in nutrient availability.

Results

Soil nutrient content and nutritional status of *P. pinaster* stands in NW Spain

The *P. pinaster* reforestation stands were established on typical Galician forest soils, characterized by a sandy texture, acidic pH, high contents of organic matter, and low levels of nutrients, especially of available phosphorus (Table 3). Total nitrogen contents were high in almost all sites, showing a more reduced variation than the other macronutrients. On the contrary, available P, and exchangeable Ca and Mg were extremely variable, with coefficients of variation over 100%. The discrepancy between the mean and the median for these three nutrients indicates skewed distributions, with most of the sites having low

253 nutrient concentrations, and only a few having extreme high values. For instance, in the
254 case of P, despite a mean value of 20.6 mg kg^{-1}, 16 out of the 22 stands had P values below
255 10 mg kg^{-1}. These results reflect the diversity of previous land uses on the studied sites.
256 Most of the sites were typical forest sites or marginal agricultural lands where agriculture
257 was abandoned many years ago, but some sites were used for crop production until very
258 recently, with organic and inorganic fertilization and intensive tillage favouring higher
259 nutrient contents. Soil characteristics in the three family × fertilization trials were within
260 the range of the reforestation plots (Table 3).

261 None of the 22 reforestation stands and the 3 family × fertilization trials showed
262 foliar concentrations above the respective critical levels for all macronutrients, indicating
263 that all plots suffer some degree of nutrient deficiencies (Figure 2). The most frequent
264 deficiencies were in P and Mg, with 16 and 18 out of the 22 stands showing severe
265 deficiencies, respectively. K and Ca foliar concentrations were below the critical level in
266 11 and 7 cases, respectively. On the other hand, all reforestation stands showed satisfactory
267 foliar nitrogen levels except three sites. These high foliar nitrogen concentrations were
268 reflected in a large imbalance between nitrogen and phosphorus nutrition with 20 out of the
269 22 stands with N:P ratios above the critical threshold of 12.5.

270 The three family × fertilization trials showed similar patterns of foliar nutrient
271 concentrations, with satisfactory levels of nitrogen but marginal or critical levels of the
272 other four macronutrients (Figure 2).

273 Phosphorus was the only nutrient for which foliar concentrations were significantly
274 correlated with soil nutrient levels ($r = 0.45$, $p < 0.05$, $N = 22$). In addition, pine growth in
275 the reforestation stands was significantly correlated with foliar concentrations of P ($r =$
276 0.42, $p < 0.05$) and Ca ($r = 0.47$, $p < 0.05$).

277

Fertilization response in family × fertilization trials

Fertilization treatments significantly increased pine height growth in the three family × fertilization trials (Table 4), but this positive effect decreased with time (Figure 3). The fertilization × site interaction was strong and significant in all the years (Table 4), indicating that the fertilization treatments had different effects on height growth depending on the trial. Site B showed the strongest response to the fertilization treatments, with an average response ranging from 30% one year after the application of treatments to 15% five years later (Figure 3A). The average response to the fertilization treatments in the other two sites was much lower and became not significant with time (Figure 3A).

The specific responses to the fertilization treatments in site B were similar between treatments, and the response to the best treatment (Figure 3B) was not very different to the average response to all the fertilization treatments (Figure 3A). Accordingly, specific contrasts revealed no significant effects of single nutrient additions in this site (Figure 4). In the other two sites, the effect of some specific nutrients on pine growth was especially strong. The addition of PCa, K, or Mg significantly increased height growth in site A, as did K in site C, whereas the inclusion of N reduced growth in both sites, and adding P significantly reduced growth in site C. These variable effects of adding specific nutrients resulted in large differences in these two test sites between the maximum response to the best treatment (Figure 3B) and the average response to all the fertilization treatments (Figure 3A). The maximum response was obtained for the F2 treatment (PCa+K+Mg) in sites A and B, and for F3 (N+K+Mg) in site C.

Genetic variation in the response to fertilization

Differences among families were significant for height growth over the five years after planting with no clear trend over time (Table 4). Families also differed significantly in their response to the fertilization treatments, as revealed by the significant family × fertilization

304 interaction (Table 4). Furthermore, the family × site interaction and the family ×
305 fertilization × site were significant too, indicating that both the family growth and the
306 family response to fertilizers varied between sites.

307 The significant family × fertilization interaction suggests a genetic variation in the
308 response to nutrient availability within the studied population. To test for the genetic
309 control of this response we analysed the variation of the $RDPI_s$ plasticity index estimated
310 for each genotype and test site by means of ANOVA. With our experimental layout we
311 were unable to detect genetic differences in the response to nutrient availability, but we
312 found a strong environmental effect (Table 5).

313 According to this weak genetic control of the response to fertilization, the family ×
314 fertilization interaction for height growth was relatively small when compared to the family
315 × site and the family × site × fertilization interactions. Considering the family factor and all
316 the interactions involving this factor as random effects in the general mixed model, the
317 estimate of the family × fertilization variance component was around 80 times less than the
318 other two interaction components, and 30 times less than the family variance component
319 (data not shown).

320

321 **Discussion**

322 The results of the present paper indicate that growth of *P. pinaster* in North West Spain,
323 although benefiting from favourable temperature and rainfall patterns, is limited by nutrient
324 availability. Soil nutrient concentrations were relatively low in many of the study sites.
325 Foliar nutrient concentrations were below the critical levels in most of the cases, and below
326 the values commonly observed in other *P. pinaster* stands in France and Australia that are
327 known to be nutrient limited (Warren *et al.*, 2005; Trichet *et al.*, 2008). As in other forest
328 tree species in the area, the most common deficiencies were in P and Mg, and to a lesser

329 extent in K and Ca, while N was usually well supplied. Sánchez-Rodríguez et al. (2002)
330 and Zas & Serrada (2003) reported that most of the *P. radiata* plantations studied in NW
331 Spain were deficient in P, Mg and, in some cases, in Ca, while they were satisfactory in N.
332 Similarly, high and satisfactory N foliar concentrations and low and frequently critical P,
333 K, Ca and Mg concentrations were reported for *Pseudotsuga menziesii* (Zas, 2003; Solla-
334 Gullón *et al.*, 2006) and *Eucalyptus globulus* (Merino *et al.*, 2003) in the area. In addition,
335 P was the only nutrient for which foliar concentrations significantly correlated with soil
336 contents, as commonly occurs for nutrients with limited availability (e.g. Gallardo and
337 Covelo, 2005). In agreement with this nutritional diagnosis, pine growth was significantly
338 correlated with foliar P and Ca concentrations. Similarly, early growth and/or site index has
339 been shown to be significantly correlated with foliar and soil P in *P. radiata* (Sánchez-
340 Rodríguez *et al.*, 2002; Zas and Serrada, 2003) and with foliar P and Ca in *Pseudotsuga*
341 *menziesii* (Zas, 2003) in NW Spain.

342 According to the deficiencies detected through foliar nutrient analysis, a significant
343 positive response to nutrient additions was observed in the three family × fertilization trials,
344 although these responses varied from site to site. *P. pinaster* response to fertilization has
345 been reported before (e.g. Warren *et al.*, 2005; Trichet *et al.*, 2008) but little information
346 was available for NW Spain, where it is generally accepted that this species responds little
347 to nutrient additions (Bará, 1990). In sandy acidic soils in France (Saur, 1990; Trichet *et*
348 *al.*, 2008) and Australia (Warren *et al.*, 2005), large growth increments, comparable to
349 those found here, have been reported in response to nutrient additions, especially of P, and
350 superphosphate fertilization is now routinely used in those areas.

351 Response to fertilization has been also documented in NW Spain for other fast
352 growing species such *P. radiata* (Solla-Gullón *et al.*, 2004; Zas *et al.*, 2006a). In a similar
353 family × fertilization trial series as the one studied here, *P. radiata* showed large responses
354 to P and Mg additions, with height increments larger than those observed here for *P.*

355 *pinaster* (up to 170% over the unfertilized control one year after fertilization, Zas et al.
356 2006a). In addition, the response of *P. radiata* to different nutrient combinations was very
357 similar across sites (Zas *et al.*, 2006a), whereas here the response of *P. pinaster* was very
358 variable, despite similar foliar nutritional diagnosis in the three trials. Thus, the response to
359 fertilization agreed with the soil nutrient levels and foliar nutrient diagnosis in some cases
360 but not in others. In site B all nutrients except N were below the critical levels. This was
361 the site where the overall response to fertilizers was higher and no significant differences
362 were detected for specific nutrient additions. In site A, the significant response to PCa, K,
363 and Mg, and the negative effect of N agreed with the foliar nutrient diagnosis for these
364 nutrients. Similarly, N additions have been shown to have a negative effect in *P. radiata*
365 fertilization trials (Zas *et al.*, 2006a), where a large imbalance between N and P and
366 between N and K has been reported (Zas and Serrada, 2003). The negative effect of N
367 fertilization was also observed in site C, where N foliar concentrations were again above
368 the critical value. However, in this site, the negative effect of P fertilization was
369 unexpected given the low foliar P concentrations and the high foliar N:P ratio in this site.
370 The negative effect of P in this site can be explained by the incidence of a *Hylobius abietis*
371 (Curculionidae, Coleoptera) attack during the first two years after planting that disturbed
372 the normal response to fertilization. The pine weevil preferentially attacked the plants
373 fertilized with PCa, causing large height reductions that outweighed the growth gains
374 generated by fertilization (Zas *et al.*, 2006b).

375 The response to fertilization not only varied among sites, but also among genetic
376 entries. The significant family × fertilization interaction suggests that the ability to respond
377 to fertilization varied among families, i.e., the phenotypic response or family plasticity to
378 nutrient availability was under genetic control. Theoretically, phenotypic plasticity should
379 be measured at the genotypic level, so the effects of genetic variability can be distinguished
380 from variation in phenotypic expression (Pigliucci, 1996). Because families are not single

genotypes, but a mixture of related genotypes with the same mother tree, the value of a given family in a given environment can incorporate some uncontrolled genotypic effects as well as some genotype × environment interaction. These uncontrolled effects may disturb the estimation of actual phenotypic plasticity, so the variation in the family response to nutrient addition is not, strictly speaking, a direct measure of variation in phenotypic plasticity. Ideally, to estimate the genetic control of phenotypic plasticity, individual trees belonging to different families should have been clonally replicated in the different fertilization treatments. Then the family structure of the sample could be used to estimate the genetic control of plasticity. Although it is well known that there is abundant genetic variation for plastic responses (Pigliucci, 2005), there is very little information based on proper empirical approaches with clonally replicated genotypes allowing the quantification of this variation and the estimation of heritability of plastic responses in strict terms, especially for forest trees (Chambel *et al.*, 2005). With our experimental design, however, we can have several estimates of the sensibility of each family to nutrient availability variation, allowing us to statistically quantify the genetic control of the family responsiveness to fertilization. We found little or no genetic variation for the plastic responses at the family level to nutrient additions across environments. The response to fertilization seemed to be strongly influenced by other environmental factors that differentially modulate the plastic response of each family in each environment. This could be easily due to non linear reaction norms and different nutritional soil conditions among the test sites. Adding the same amount of nutrients to soils differing in nutrient availability and fertilization efficiency, may have led to variations in the family response to fertilizers (Namkoong *et al.*, 1992). In accordance with this, although soil and foliar diagnosis of the three test sites were quite similar, response to fertilization greatly varied among sites, and this variation altered the family response to the fertilization treatments, as revealed by the significant family × fertilization × site interaction term. In addition, the genetic response to

the fertilization treatments could also vary among sites because of the influence of other unknown biotic and/or abiotic environmental factors that varied among the test sites. In instance, different symbiotic fungal communities among sites may differentially alter the family responses to nutrient additions in different sites (Mari *et al.,* 2003). Moreover, the ability to get infected by specific mycorrhizal species may differ between pine families, as observed in other conifer species (Korkama *et al.,* 2006). Besides, interactions with other environmental factors are known to alter the plastic response to a specific environmental factor such is the case, for example, with the plastic responses to shade depending on the water availability (Climent *et al.*, 2006; Sánchez-Gómez *et al.*, 2007).

Our results suggest that, although there was genetic variation for the response to nutrient availability, this variation was strongly environmentally modulated, hampering both the evolution of specific responses as adaptations to local environmental conditions, and the possibility of artificially selecting, within breeding programs, genotypes suited to different soil conditions and/or silviculture regimes. Further research, comparing the plasticity of *P. pinaster* populations coming from different soil conditions, would help to understand the hypothesized local adaptations to low and heterogeneous nutrient availability of the NW maritime pine population (Bradshaw, 2006). The large population divergence in the phenotypic response of *P. pinaster* to water availability, with the Atlantic provenance of Leira (Portugal) being the most plastic origin (Chambel *et al.*, 2007), is an attractive start point for this investigation.

In summary, nutritional disorders of *P. pinaster* in NW Spain were frequent and similar to those found in other fast growing species, but the qualitative response to fertilization was not as clear as that found for other species, specifically in radiata pine (Zas *et al.*, 2006a). Judging from the results presented here, P and Mg fertilization should be recommended to increase early growth and attain crown closure earlier, but the lack of a consistent response among sites, and the possible undesired effects of fertilization, such as

the increase in pest incidences (Zas *et al.*, 2006b) or the increase in susceptibility to storm damage (Cucchi and Bert, 2003; Trichet *et al.*, 2008), suggest that the convenience of fertilization cannot be generalized and it should be specifically determined in each case. The results also indicated that families differed in the response to the fertilization treatments but this family response was environmentally dependent. The strong environmental component modulating the phenotypic responses to nutrient availability in nutrient-lacking soils could impose an important obstacle for the selection towards local adaptations to low and heterogeneous nutrient availability. Analogously, it hinders the artificial selection of genotypes adapted to produce high productivity phenotypes under different soils and/or fertilization regimes.

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583 FIGURE CAPTIONS
584
585 Figure 1. Location of the 22 reforestation plots (black dots) and the three family ×
586 fertilization trials (grey squares, A, B, and C). The shaded area represents the current

587 distribution of *P. pinaster* in Galicia derived from the Third National Forestry Inventory
588 (DGCN, 2002).
589
590 Figure 2. Foliar nutrient concentration in the 22 *P. pinaster* reforestation stands (dots) and
591 in the 3 family × fertilization trials (triangles A, B and C)). Each data point represents the
592 mean of 2-3 composite foliar samples of 5-10 trees each. Dot and triangle colours indicate
593 the nutrient diagnosis according to the critical values reported by Bonneau (1995): white =
594 satisfactory levels, grey = marginal levels, and black = critical levels.
595
596 Figure 3. Effect of fertilization on height growth in three *P. pinaster* family × fertilization
597 trials during the first five years after establishment and fertilization application. Overall
598 mean relative response (panel **A**) of eight different fertilization treatments (see Table 2)
599 relative to the unfertilized control, and maximum relative response to the best fertilization
600 treatment observed at each site (panel **B).**
601
602 Figure 4. Estimates of the effect of single nutrients (N, PCa, K, and Mg) on *Pinus pinaster*
603 height in three family × fertilization trials five years after establishment and treatment
604 applications. Each bar represents the estimate of the effect of including every specific
605 nutrient in the fertilization treatment obtained from specific contrasts in the mixed model.
606 The linear functions for these contrasts were as follows (see Table 2 for treatment
607 description): (F_1, F_3, F_5, F_7) vs. (F_2, F_4, F_6, F_8) for estimating the effect of N addition, (F_1,
608 F_2) vs. (F_3, F_6) for P, (F_1, F_2) vs. (F_4, F_7) for K, and (F_1, F_2) vs. (F_5, F_7) for Mg. Significant
609 effects are denoted by * ($p<0.05$), ** ($p<0.01$), or *** ($p<0.001$).
610