RESEARCH PAPER

Long-term effect of fertilization on stone pine growth and cone production

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Abstract

· **Key message Fertilization is a feasible management technique for *Pinus pinea* L. cropping, with increased cone production up to 9 years after the application and superior trunk diameter growth every year. Fertilization effects on cone size-weight indicate the value of re-fertilizing.**

· ***Context*** Stone pine (*Pinus pinea*) is a species of economic interest for its highly in demand pine nuts. Despite the high value of pine nuts, cones are harvested mostly from non-managed forests. Consequently, advances in cropping techniques, such as fertilization, are needed.

· ***Aims*** To monitor the effect of fertilization on growth and cone production during 10 years after fertilization, adjusted to soil characteristics, on a 16-year-old stone pine orchard.

· ***Methods*** A fertilization trial including fertilized and non-fertilized plots was established. Diameter (trunk and crown) and height were repeatedly measured in each tree for 10 years. Cones were annually harvested from each tree and counted.

· ***Results*** Stone pine cone production increased significantly from the third to the ninth year of treatment, with a peak increase of 3.3 times.

· ***Conclusion*** Fertilization was a useful silvicultural practice to increase stone pine cone production. Periodical fertilization is recommended.

Keywords Stone pine · Fertilization · Cone production

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1 Introduction

Stone pine (*Pinus pinea* L.) is a valuable species for its delicious and highly nutritious edible seeds, the pine nuts or pinoli (Segura et al. 2006), the most expensive nut worldwide (INC 2018). Pine nuts are a good source of fat, proteins, vitamins (E, B6, niacin, folic acid), and a variety of phytochemicals, and contain high levels of potassium, phosphorus, magnesium, zinc, iron, and copper (Nergiz and Dönmez 2004; Evaristo et al. 2010; Bolling et al. 2011; Lutz et al. 2016). Despite the high value of pine nuts, specific cultivation techniques of stone pine as a fruit tree have been poorly developed, since cones are mostly harvested from natural forests (Mutke et al. 2012).

Stone pine is mostly distributed in coastal areas (Vendramin et al. 2008), which are considered one of the best sites for growth (Bussotti 1997; Campelo et al. 2007; Manzanera et al. 2016); in the coasts of Italy and Spain, soils are alluvial, volcanic (Costantini et al. 2004), or littoral dunes (Muñoz and Gracía 2009; Piraino et al. 2012). However,

along the coastal areas of southern Latin America, *P. pinea* has been planted frequently on poor, light textured soils, with mineral deficiencies, usually of boron and phosphorus (IREN-CORFO 1964; INTA 2018). The reported variation in diameter and height growth between and within plantations confirms that growth of this species is sensitive to soil conditions (Court-Picon et al. 2004; Mutke et al. 2007; Loewe-Muñoz et al. 2019a). Evaristo et al. (2010) reported significant differences in the mineral profile of pine nuts grown in different regions, suggesting that environment, and particularly soil types, could have an important influence. In the Southern Hemisphere, Vanhanen and Savage (2013) also reported variability in minerals probably related to soil type and nutritional status.

Fertilization is one of the most important management techniques to adjust soil nutrients, promoting growth and improving fruit yield and quality in fruit trees (Marschner 2012; Sansavini and Ranalli 2012). A positive impact of fertilization, especially in terms of nitrogen availability, has been reported for pines such as *P. pinaster* (Zas and Fernández-López 2005), *P. edulis*, and *P. monophylla*, with records of enhanced cone production (McLain 2008). Fertilization was shown to be a positive silvicultural practice in *P. taeda*, enhancing growth, even with low water availability (Maggard et al. 2016).

The short-term effect of fertilization on stone pine growth and fruiting was confirmed in an area of poor soil in Chile; after two consecutive years of fertilization, significant positive effects on diameter and height growth, as well as on 1-year-old conelet production, were observed (Loewe et al. 2017). However, unlike in other fruit tree species, long-term effects of fertilization on stone pine are unknown. Studies in this species are challenging because cone development takes 42 months from differentiation to harvest, and 1-, 2- and 3-year-old cones occur simultaneously on the same tree, making the evaluation of any management practice more complex than in other species (Loewe et al. 2016). The known positive correlations of diameter at breast height (DBH) and crown diameter with cone number in stone pine suggest that, if vegetative growth increases as a consequence of fertilization, pine nut production may also increase. Long-term studies are needed to better understand the duration of fertilization effects on cone production (cone number and size-weight). This type of basic information is crucial to define the re-fertilization requirements of stone pine and fertilization management schedules.

Fertilization of soils with unbalanced mineral content is more complicated than that in sites of medium to high fertility (Kolari 1983). The effect of soil fertilization including nitrogen, phosphorus, and potassium is expected to be positive in forests with no deficiency of micronutrients, especially boron (Paavilainen 1990). The impact of micronutrients on stone pine has only been addressed by Bento and Coutinho (2011), and Malchi and Shenker (2011), who studied boron

and iron deficit, respectively. Nutrient deficiency affects stone pine fruit production. In poor soils of the species' native area, nitrogen, phosphorus, calcium, and manganese depletion was positively correlated with needle and cone loss (Kilci et al. 2013). In Portugal, Freire et al. (2019) reported that the most productive stone pine stands (up to 6000 kg cones ha^{-1} year^{-1}) correspond to agroforestry systems associated with fertilized pastures, indicating the convenience of soil fertilization to obtain not only more cones, but also heavier ones. In Spain, Calama et al. (2007) monitored cone production and quality in an adult (50 year old) stone pine forest and found a positive response, although lower than expected, to fertilization with a high dose of dolomite (calcium magnesium carbonate); the authors suggested exploring the effects of nitrogen fertilizers.

To the best of our knowledge, no long-term studies monitoring the effect of fertilization on stone pine cone production have been published. The hypothesis to be tested was that fertilization of stone pine plantations, established on nutrient poor soils, would boost vegetative growth and cone production, but that this effect would not be long-lasting. The objective of this work was to monitor growth and production during several years after fertilization, adjusted to account for the existing nutritional status of the soil, on a 16-year-old stone pine orchard.

2 Material and method

2.1 Experimental trial

The trial was conducted in a 2-ha, 16-year-old non-irrigated stone pine plantation located in Toconey (35° 24' S, 72° 3' W), Maule region, Chile, on smooth hills with soil of granitic origin. The climate in the region is typically Mediterranean, characterized by long dry summers and short and intense rainfalls in winters, with maritime influence given by the entrance of the Pacific Ocean fog into the valley through the Maule River. Average annual climate data (2009 to 2018) for Toconey shows rainfall of 630 mm, evapotranspiration of 1451 mm, 8 dry months year^{-1} [dry month = (monthly rainfall/monthly evapotranspiration) < 0.5], and average temperature of 14.2 °C (www.dga.cl). The climatic variables related to stone pine productivity for the study period are summarized in Table 1 and were taken from Loewe et al. (2016).

A soil survey was conducted across the plantation area following a systematic zigzag pattern in three zones randomly located; at each sample point, soil from three depths (up to 60 cm) was collected to build a composite sample. Soil analyses (Table 2) indicated that the trial site is on a silty, non-salty soil with neutral pH, and medium organic matter content. Mineral composition showed a very low phosphorus, zinc, sulfur, and boron content; low calcium content; and medium

Table 1   Climate variables influencing stone pine cone production in Toconey, Chile, during the study

Year	Annual rainfall (mm)	Annual thermal oscillation† (°C)	Autumn thermal oscillation (°C)	Spring rainfall (mm)	Spring Hydric Index†† (mm)
2009	689.0	16.2	14.6	66.4	−385.3
2010	566.0	16.4	15.9	28.9	−441.8
2011	631.0	15.4	13.6	15.8	−435.2
2012	543.0	15.2	14.1	82.4	−333.5
2013	508.0	16.6	15.9	26.4	−455.2
2014	839.0	15.9	13.8	41.1	−379.1
2015	691.3	13.9	12.9	130.9	−285.7
2016	484.0	14.1	10.9	71.3	−398.0
2017	808.0	14.4	12.9	95.2	−343.9
2018	543.0	14.7	14.1	83.3	−367.7
Average	630.2	15.3	13.9	64.2	−382.5

† Thermal oscillation: average maximum absolute temperature minus average minimum absolute temperature for a given period expressed in degrees Celsius

†† Hydric index, an indicator of water deficit, HI: rainfall minus potential evapotranspiration, expressed in mm

nitrogen, potassium, magnesium, copper, iron, and manganese content.

In winter 2009, before the start of the assay, management practices were applied homogeneously with the aim of obtaining a strong, well-formed, productive tree crown in the orchard. Trees were trained with one main leader and the trunk hand-pruned on the lower three meters, removing all lateral branches. A thinning was applied in the orchard, which consisted of cutting down 50% of trees, leaving on average 204 trees/ha. Two treatments were established, involving

Table 2   Soil properties at the trial site (Toconey, Chile)

Property	Toconey site	Method
Texture (%)		Bouyoucos soil hydrometer (USDA 2004)
Sand	29 ± 0.00	
Lime	45 ± 0.00	
Clay	26 ± 0.00	
pH	6.03 ± 0.18	Glass electrode ph meter (1:2.5 soil to water suspension) (Sadzawaka et al. 2006)
Salinity, EC (mmhos cm^{-1})	0.04 ± 0.00	Electrical conductivity of the saturated paste extract (1:1 soil to water) (Sadzawaka et al. 2006)
Organic matter (%)	2.27 ± 0.18	Oxidation and colorimetry (Sadzawaka et al. 2006)
Nutrient content		
N (mg kg^{-1})	21.33 ± 6.06	Extraction with kcl 2 mol l^{-1} (Sadzawaka et al. 2006)
P (mg kg^{-1})	3.33 ± 0.88	Extraction with nahco$_3$ 0.5 mol l^{-1} at ph 8.0 (Sadzawaka et al. 2006)
K (mg kg^{-1})	91.33 ± 11.10	Extraction with nh$_4$ch$_3$co$_2$1 mol l^{-1} at ph 7.0 (Sadzawaka et al. 2006)
Ca exch (cmol+ kg^{-1})	88.03 ± 85.48	Extraction with nh$_4$ch$_3$co$_2$ 1 mol l^{-1} at ph 7.0 (Sadzawaka et al. 2006)
Mg exch (cmol+ kg^{-1})	0.79 ± 0.02	Extraction with c nh$_4$ch$_3$co$_2$ 1 mol l^{-1} at ph 7.0 (Sadzawaka et al. 2006)
Cu (mg kg^{-1})	0.50 ± 0.15	Extraction with dtpa (diethylenetriaminepentaacetic acid) (Sadzawaka 1990)
Zn (mg kg^{-1})	0.22 ± 0.05	Extraction with dtpa (diethylenetriaminepentaacetic acid) (Sadzawaka 1990)
Mn (mg kg^{-1})	16.47 ± 7.03	Extraction with dtpa (diethylenetriaminepentaacetic acid) (Sadzawaka 1990)
Fe (mg kg^{-1})	23.33 ± 2.85	Extraction with dtpa (diethylenetriaminepentaacetic acid) (Sadzawaka 1990)
B (mg kg^{-1})	0.31 ± 0.10	Extraction with cacl$_2$ mol l^{-1} (Sadzawaka 1990)
S (mg kg^{-1})	< 2	Extraction with ca (h$_2$po$_4$)$_2$ 0.01 mol l^{-1} (Sadzawaka et al. 2006)

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fertilized and non-fertilized plots, following a completely randomized design with six replicates for the former treatment and two replicates for the latter. The experimental units were plots of 25 and 10 trees for fertilization and non-fertilization treatments, respectively.

In spring 2009 (at plantation age of 16 years), fertilization was applied equally in all fertilized plots, including macronutrients (phosphorus, potassium, calcium, and sulfur) and micronutrients (boron and zinc) at rates determined according to the soil deficiencies identified through the soil analyses. Fertilizer was supplied at 260 g per tree, and was composed of phosphoric acid (60 g), calcium phosphate (60 g), zinc sulfate (10 g), sodium borate (20 g), potassium sulfate (60 g), and carbamide (50 g). Doses of the applied elements per tree were nitrogen (23 g), phosphorus (49 g), potassium (31 g), boron (4 g), zinc (2 g), calcium (12 g), sodium (2 g), and sulfur (18 g). Fertilizer was applied at 5 cm soil depth on two lateral strips located at the edge of the crown projection.

2.2 Recorded data

Growth was repeatedly measured in each tree during the 2009–2018 period; diameter at breast height (DBH) was recorded at 130 cm above the ground with a caliper (Haglöf, Långsele, Sweden) and tree height, with a hypsometer (Suunto, Vantaa, Finland). At the end of the study period, vigor was assessed in all trees using a categorical scale: (1) high vigor (abundant foliage, dark green needles), (2) medium vigor (abundant to regular foliage, light green to yellow needles), and (3) low vigor (scarce foliage, strong discoloration), based on Lakatos and Mirtchev (2014). All 3-year-old cones were annually harvested and counted in July from 2009 to 2018. Harvested cones were classified according to the following categorical scale: small (< 350 g), medium (350–550 g), and big (> 550 g); 350 and 550 g were the first and third quartile, respectively, of the weight distribution, which was determined by a sample of 100 randomly selected cones. Analyses were performed using data from the 2012–2018 period to avoid the impact of management practices (pruning and thinning) applied at the beginning of the study.

Cone health was monitored in each harvested cone through visual observation; cones were classified as healthy (without any external damage) or damaged (presence of at least one of the following symptoms: holes, excessive accumulation of resin, deformations).

2.3 Statistical analysis

An ANOVA for repeated measurements was fitted for each variable using a model with the following terms: treatment effect, year effect, treatment × year interaction regarded as fixed effects, and a random plot effect. Normal distribution was assumed for the error terms of the models for height,

DBH and crown diameter, and a Poisson distribution for the total number of 3-year-old cones per tree and the number of mature cones in each cone weight category. Two new variables were created from the measurements of cone number tree^{-1}: cone number per crown area (C/C, cones m^{-2}) and cone number per trunk diameter (C/DBH, cones cm^{-1}). DBH in 2009 was used as covariate to adjust for size variability among trees in all fitted ANOVA models. Tree vigor was analyzed through the percentage of high vigor trees per plot at the end of the study assuming a binomial distribution. Cone health was not statistically modeled because all cones were healthy. Statistical analyses were performed using the software InfoStat (Di Rienzo et al. 2019) and its interface with the software R (www.r-project.org) to assess statistical significance of each model effect in a generalized linear mixed model framework (Stroup 2012).

3 Results

3.1 Growth

The effect of treatment × year interaction was statistically significant for DBH ($p < 0.05$), but not for crown diameter or height ($p > 0.05$) (data not shown). The yearly mean comparisons showed that the only difference in growth corresponded to DBH 9 years after fertilizer application (see results in Table 3). Average DBH growth rates for the years 2012 to 2018 of fertilized plots were higher than those of the non-fertilized ones (1.23 cm year^{-1} vs 0.87 cm year^{-1}, respectively) ($p = 0.0007$). In the same period, crown diameter (CD) and total height of trees were higher in fertilized plots, but the effect was not statistically significant ($p > 0.05$).

At the end of the study period, no statistical differences were found for vigor between fertilized and non-fertilized trees, given that 90 to 95% of trees were classified as vigorous in all plots.

3.2 Cone production

The number of harvestable cones per tree showed a statistical significant increase in the fertilized trees between the third and ninth years after treatment, with a peak in the fifth year (2014)—when fertilized trees had 3.3 times more cones than non-fertilized ones (Table 3 and Fig. 3. in Annex). Cone number tree^{-1} showed synchronous variability over the years. When cone number tree^{-1} was expressed in relation to trunk diameter (C/DBH), the effects of the treatment × year interaction and of treatment were not statistically significant ($p > 0.05$) (Table 3). However, cone number tree^{-1} expressed as unit of crown area showed statistical differences among treatments 5 years after fertilization and at the end of the study ($p < 0.05$) (Table 3).

Table 3 Stone pine growth and cone number for each treatment after 3 to 9 years of fertilization

Variable	Units	Treatment	Year						
			2012	2013	2014	2015	2016	2017	2018
DBH	[cm]	Fertilized	31.2 ± 0.4 a	33.1 ± 0.4 a	34.4 ± 0.4 a	35.7 ± 0.4 a	36.7 ± 0.4 a	37.8 ± 0.4 a	38.8 ± 0.4 a
		Non-fertilized	30.5 ± 1.0 a	32.4 ± 1.0 a	33.1 ± 1.0 a	34.8 ± 1.0 a	35.6 ± 1.0 a	35.9 ± 1.0 a	36.2 ± 1.0 b
CD	[m]	Fertilized	6.6 ± 0.1 a	7.2 ± 0.1 a	7.2 ± 0.1 a	7.2 ± 0.1 a	7.4 ± 0.1 a	7.5 ± 0.1 a	7.7 ± 0.1 a
		Non-fertilized	6.3 ± 0.3 a	7.1 ± 0.3 a	7.1 ± 0.3 a	7.1 ± 0.3 a	7.1 ± 0.3 a	7.2 ± 0.3 a	7.3 ± 0.3 a
Height	[m]	Fertilized	11.9 ± 0.2 a	12.7 ± 0.2 a	12.7 ± 0.2 a	12.7 ± 0.2 a	13.6 ± 0.2 a	14.5 ± 0.2 a	15.4 ± 0.2 a
		Non-fertilized	11.5 ± 0.5 a	12.1 ± 0.5 a	12.4 ± 0.5 a	12.7 ± 0.5 a	13.5 ± 0.5 a	14.3 ± 0.5 a	15.1 ± 0.5 a
Cones	[#]	Fertilized	4.83 ± 0.9 a	14.6 ± 2.6 a	14.0 ± 2.5 a	9.8 ± 1.8 a	10.1 ± 1.8 a	20.1 ± 3.6 a	17.8 ± 3.2 a
		Non-fertilized	2.57 ± 0.9 a	7.2 ± 2.3 a	4.3 ± 1.4 b	3.9 ± 1.3 b	4.1 ± 1.3 b	9.5 ± 3.0 b	7.5 ± 2.4 b
C/DBH	[#/cm]	Fertilized	0.18 ± 0.08 a	0.50 ± 0.08 a	0.45 ± 0.08 a	0.30 ± 0.08 a	0.31 ± 0.08 a	0.62 ± 0.08 a	0.55 ± 0.08 a
		Non-fertilized	0.09 ± 0.16 a	0.21 ± 0.16 a	0.12 ± 0.16 a	0.10 ± 0.16 a	0.11 ± 0.16 a	0.26 ± 0.16 a	0.21 ± 0.16 a
C/C	[#/m^2]	Fertilized	0.16 ± 0.05 a	0.38 ± 0.05 a	0.35 ± 0.05 a	0.24 ± 0.05 a	0.24 ± 0.05 a	0.48 ± 0.05 a	0.42 ± 0.05 a
		Non-fertilized	0.10 ± 0.10 a	0.20 ± 0.10 a	0.12 ± 0.10 b	0.10 ± 0.10 a	0.11 ± 0.10 a	0.25 ± 0.10 b	0.19 ± 0.10 b

Mean ± SEM. By year, among variables and treatments, different letters indicate statistical differences ($p < 0.05$)

DBH, diameter at breast height (1.3 m); *CD*, crown diameter, defined as the width of the crown; *Height*, total tree height; *Cones*, all cones from each tree were manually harvested to ensure that the entire production was assessed; *C/C*, cone number per crown area (cones m^{-2} tree^{-1}); *C/DBH*, cone number per trunk diameter (cones cm^{-1} tree^{-1})

Regarding cone size-weight categories, the fertilization effect peaked 5 years after the intervention, declining thereafter. In fact, differences in percentage of big and medium cones between treatments were observed, with an increasing proportion of medium and small cones for the fertilized plots over time (Table 4 in Annex). Figure 1 depicts the differences in cone size-weight category distribution between 2014 and 2018. In 2014, the higher number of cones in fertilized plots was distributed in big and medium cones (Fig. 1 top). However, at the end of the study, fertilized plots had a higher number of cones than non-fertilized ones, but cone distribution by the size-weight category indicated a higher presence of small cones than 5 years after fertilization (Fig. 1 bottom).

The estimated cone production per hectare shows a difference of over 1500 kg between fertilized and non-fertilized plots in 2017, the bumper crop year (Fig. 2). Regarding cone health, all cones were classified as healthy.

4 Discussion

In our study, regarding vegetative growth, fertilized trees exhibited an increase in average diameter growth rates in years after treatment of over 40% with respect to non-fertilized trees, showing that fertilization has a positive effect on stone pine trunk diameter, as reported by Kilci et al. (2013). This positive effect of nutrient supplementation on a plantation established in a poor soil is consistent with the important nitrogen uptake reported for similar soils in a stone pine forest located in the coast of France (Rapp et al. 1979). Reports for other pines, such as *P. tropicalis*, indicate that nutritional deficiencies affected diameter growth, with P being the main limiting nutrient (Ferrer et al. 2004); moreover, for *P. radiata*, boron deficiency was reported to limit growth, cause malformations, and inhibit fruiting (Schlatter and Gerding 1984). Low content of micronutrients, particularly of boron, might limit the expected effect of nitrogen supplementation on vegetative growth (Paavilainen 1990). However, the simultaneous supply of macro- and micronutrients in the studied plantation favored stem growth, even though the soil had a very low boron content. Contrary to findings reported by Ravazi et al. (2006), we did not find significant effects of fertilization on height growth or crown diameter, which could be due to the limited space available for crown expansion in the current average spacing (7 × 7 m).

In order to promote adaptation to climate change, management practices oriented to achieve the highest possible tree vigor are desirable to reduce susceptibility to pathogen attacks and drought episodes. Moreover, vigor is correlated to number of female flowers or strobili (Mutke 2005). At the end of our study, 9 years after fertilization, there were no differences in vigor between fertilized and non-fertilized plots.

Cone production showed high variability throughout the years; this is a typical behavior of the species, which is characterized by a masting habit (Prades et al. 2005). The observed synchronicity of cone production throughout the study period, regardless of the nutritional status, suggests that this
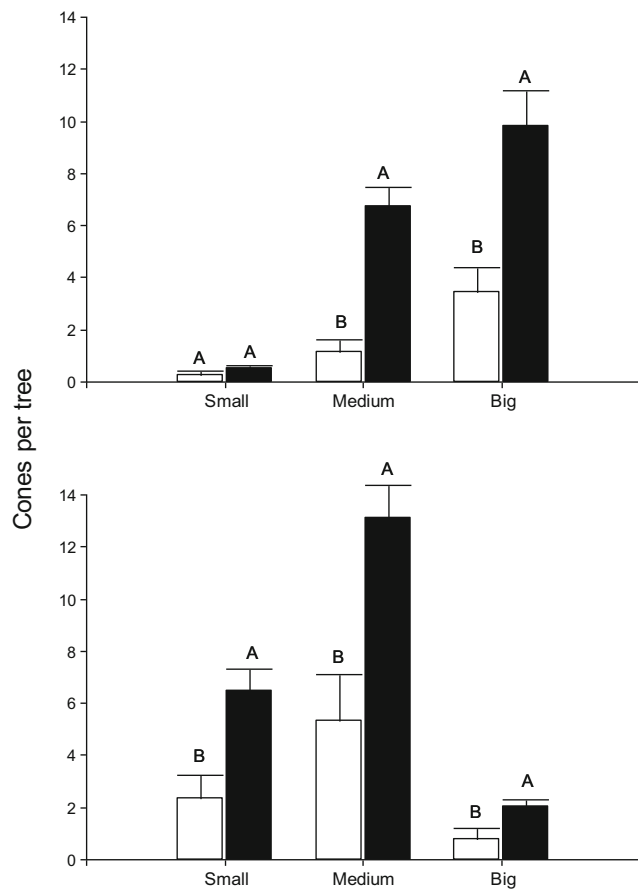
Fig. 1 Cones per tree and size-weight category in fertilized (*black*) and non-fertilized (*white*) trees five (2014) (*top*) and nine (2018) (*bottom*) years after fertilization in Toconey, central Chile. Different letters within a weight category indicate statistical differences between treatments in cone number per tree for that category ($p < 0.05$)

variability is a response to climate conditions. Indeed, Calama et al. (2019) and Loewe et al. (2016) found that cone
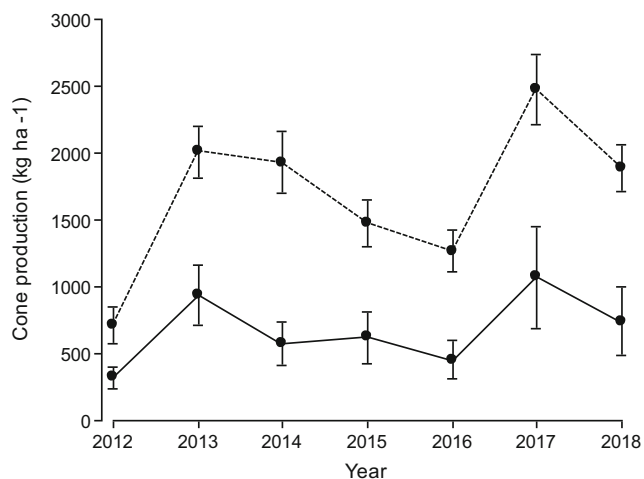
Fig. 2 Evolution of cone production per hectare in fertilized (*dashed line*) and non-fertilized (*solid line*) plots 3 to 9 years after fertilization (2012 to 2018) in a stone pine plantation located in Toconey, central Chile. Mean ± SE

production in Spain and Chile, respectively, was favored by an annual thermal oscillation below 12 °C, among other climatic factors. This climatic condition does not occur in the study site, and would explain the lower cone production than in other areas of Chile (Loewe et al. 2016). Despite that inter-annual variability, our fertilized plots maintained a significantly higher production than non-fertilized ones. Other authors have reported that early spring fertilization would favor female cone induction in *P. elliotti* (Hoekstra and Mergen 1957; Shoulders 1968), and male cone production for *P. radiata* (Codesido and Merlo 2007).

A positive impact of the tested spring fertilization applied in 3-year-old cone number tree^{-1} in 2009 was observed in the successive harvests from 2014, 5 years after treatment, when cone production due to fertilization was up to 3.3 times higher than in the non-fertilized plots. Our results showed a higher impact of nutrient supplementation than that reported by Kilci et al. (2013), who found that a higher dose of macronutrient fertilization (2360 g tree^{-1}) than the one used in this work (260 g tree^{-1}) resulted in a 1.4 times higher cone production than in the non-fertilized trees. The lower values obtained by Kilci et al. (2013) may be attributed to the deficiency of some micronutrients, as reported by Paavilainen (1990).

Cone production, expressed as number of cones/tree, in the first two harvests after fertilization (2012 and 2013) was not statistically significant in our study, probably because of the heavy pruning performed in 2009; indeed, 50% of the crown of many trees was extracted by removing low trunk bifurcations. After the restoration of crowns in all trees, verified after 2 years (Loewe et al. 2013), the impact of nutrient supplementation became significant and was maintained in the following harvests.

The analysis of the cone number/DBH ratio showed no significant differences between treatments over the years. The analysis in relation to crown area indicates that, also starting 5 years after the management practice and except for 2 years of low production (2015 and 2016), fertilization had a significant positive effect on cone production, enhancing the productive potential of the tree crown. Regarding cone health, we did not find differences between fertilized and non-fertilized plots; this result is in disagreement with findings of Farinha et al. (2018), who reported that cones from stone pine trees fertilized through the irrigation system are more susceptible to conelet mortality and seed damage by *Leptoglossus occidentalis*.

Although fertilization increased cone production, it did not reduce cone size-weight for 5 years after treatment, with a significant increase in medium and big cones being observed. However, at the end of the study period, the distribution of cone size-weight was skewed toward the smaller cones. Kilci et al. (2016) also reported changes in cone weight distribution in response to fertilization. Therefore, given that the effect of fertilization is not

long-lasting, periodical fertilization might be necessary to enhance cone number and size-weight, as previously reported by Calama et al. (2007), Kilci et al. (2013) and Loewe et al. (2017). Thus, re-fertilization would be necessary to obtain high cone production concentrated in bigger cones, thereby improving fruit quality (Calama et al. 2007; Freire et al. 2019). Nevertheless, a recent study concluded that pine nut production depends not only on cone number but also on pine nut number inside cones and pine nut weight (Loewe-Muñoz et al. 2019b), with number of pine nuts per cone being the most important fruit trait in determining pine nut yield. Consequently, further studies are needed to determine the effect of re-fertilization on pinoli production.

The strong and long-lasting effect of fertilization on cone number is undoubtedly a positive factor for productive stone pine stands established in silty soils of granitic origin. However, after 5 years of fertilizer supplementation, the gain in cone production decreased, which also indicates the need for repeating fertilization to enhance cone number per tree and cone size-weight distribution. The effectiveness of fertilization in increasing cone number in the long-term was not maintained for the cone size-weight category distribution. This result could be due to the higher cone size-weight of the studied categories than values reported in Portugal and Spain, where average cone weight is lower than 350 g (Gonçalves and Pommerening 2012; Mutke et al. 2012). Heavier cones would impose an important nutritional demand on the tree.

Fertilization improved cone production but, in contrast to our hypothesis, it was not a response to tree growth. The combined effect of fertilization on cone number and cone size-weight led to increases in cone production from 800 to over 1500 kg per hectare with respect to the non-fertilized treatment; this is a very interesting result from an economic perspective.

To the best of our knowledge, this is the first longitudinal study reporting the long-term impact of fertilization on stone pine cone size-weight frequency distribution, providing useful information to determine the periodicity of re-fertilization. However, these results are only directly applicable under the edaphic conditions of the trial. Further studies are needed to find out whether re-fertilization of younger and older stone pine stands can be recommended for practical forestry, as well as its effectiveness on other soil conditions.

5 Conclusions

Positive long-term effects of fertilization were found on cone production in a stone pine plantation monitored from 16 to 25 years of age. Cone number significantly increased from 5 years after fertilization, with a peak of cone production of up to 3.3 times higher than in non-fertilized trees. Fertilization effects on cone size-weight distribution showed a positive but not long-lasting effect, with a reduction in bigger cones over the years after treatment. The key finding of this work is that fertilization has a positive impact on cone production, but only for approximately 5 years. Further assays are needed to identify the best moment to repeat fertilization.

Data availability The datasets generated and analyzed during the current study are not publicly available due to institutional guidelines, but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Annexes
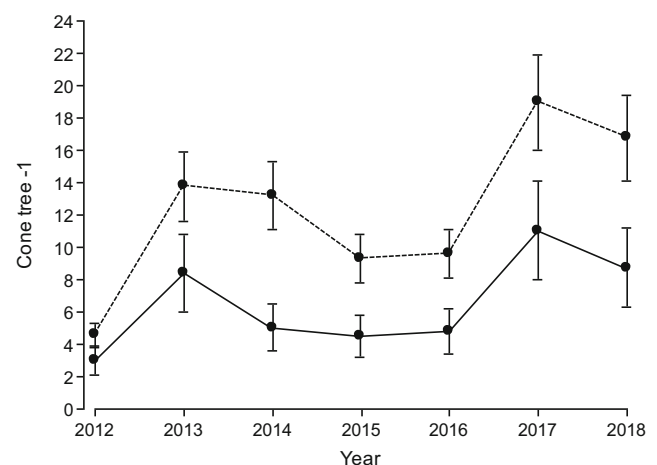
Fig. 3 Three-year-old cone production in fertilized (*dashed line*) and non-fertilized (*solid line*) trees 3 to 9 years after fertilization (2012 to 2018) in a stone pine plantation located in Toconey, central Chile. Mean ± SE

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Table 4 Stone pine cone size-weight distribution of fertilized and non-fertilized trees between 3 and 9 years after fertilization

Cone size-weight category [%]	Year						
	2012	2013	2014	2015	2016	2017	2018
Fertilized							
Big	70.0 ± 2.4 a	49.3 ± 2.1 a	56.7 ± 2.1 a	49.6 ± 2.1 a	29.4 ± 2.1 b	28.7 ± 2.0 b	8.6 ± 2.0 c
Medium	25.8 ± 2.4 b	47.0 ± 2.1 a	39.8 ± 2.1 b	41.7 ± 2.1 b	55.9 ± 2.1 a	61.8 ± 2.0 a	61.1 ± 2.0 a
Small	4.1 ± 2.4 c	3.6 ± 2.1 b	3.5 ± 2.1 c	8.7 ± 2.1 c	14.7 ± 2.1 c	9.5 ± 2.0 c	30.3 ± 2.0 b
Non-fertilized							
Big	55.9 ± 8.9 a	49.1 ± 6.8 a	67.2 ± 7.4 a	47.1 ± 7.7 a	23.2 ± 7.2 b	19.4 ± 7.0 b	11.8 ± 7.0 b
Medium	34.7 ± 8.9 a	42.9 ± 6.8 a	25.0 ± 7.4 b	38.7 ± 7.7 a	54.8 ± 7.2 a	65.6 ± 7.0 a	62.6 ± 7.0 a
Small	9.4 ± 8.9 b	8.0 ± 6.8 b	7.8 ± 7.4 b	14.2 ± 7.7 b	21.9 ± 7.2 b	15.0 ± 7.0 b	25.5 ± 7.0 b

Mean ± SEM. By year, different letters indicate statistical differences ($p < 0.05$) among cone size-weight categories within treatment

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