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Assessment of *Acacia dealbata* as green manure and weed control for maize crop

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Abstract

Acacia dealbata Link is one of the main invasive species in southwestern Europe and a resource with potential value for agriculture. Our objective was to assess the value of *A. dealbata* vegetative aerial biomass used as green manure and as a tool for weed control in maize crops through three sequential experiments. In 2017, an experiment was carried out with acacia green manure *vs* inorganic fertilization of pots sown with a field corn and a sweet corn hybrid with strong and weak nutrient demand, respectively. Nutrients were not released from acacia green manure at an appropriate timing, and maize suffered nutrient deficit. In 2018, a pot experiment was made outdoors incorporating acacia green manure at different times before maize sowing, and we found that a 4-month period was required for maximum nutrient release from acacia green manure. In 2019, an early and a late-field experiments were performed by incorporating acacia green manure 4 months before maize sowing. Physiological and agronomic data were recorded in maize, along with soil data, for all years, and weed data the last year. Altogether, most effects and interactions between genotype or environment and fertilization treatment were not significant, and some deficiencies caused by acacia green manure fertilization depend on genotype and environment. Incorporation of acacia green manure 4 months before maize sowing partially controlled weeds and replaced inorganic fertilization. However, deficiencies should be corrected with additional weed control practices and fertilization treatments, according to the nutrient demand of the crop and the soil environment.

Introduction

Subgenus *Phyllodineae* of the genus *Acacia* (subfamily Mimosaceae, family Fabaceae) provides one of the most invasive tree groups worldwide (Richardson and Rejmánek, 2011; Souza-Alonso *et al.*, 2017). *Acacia* species were introduced into new ranges for the rehabilitation of degraded lands with legume trees that have industrial uses such as tannin and pulp production, and agroforestry and ornamental uses (Griffin *et al.*, 2011). However, they escaped from original areas and have become invasive.

Among this group, *Acacia dealbata* Link (silver wattle) is one of the most pervasive acacias with increasing impacts on Mediterranean areas (Souza-Alonso *et al.*, 2017), where its invasion seriously affects ecosystems by replacing native species (Lorenzo *et al.*, 2010a, 2010c, 2012, 2017; Lazzaro *et al.*, 2014) and changing soil conditions (Lorenzo and Rodríguez-Echeverría, 2015; Souza-Alonso *et al.*, 2017). The adaptation limits for *A. dealbata* are high soil pH (pH_{CaCl_2} > 5:5), frequent frosts (>21–40 d yr^{-1}) and low annual precipitation (<500–1000 mm) (Vieites-Blanco and González-Prieto, 2020). Despite this, the invasive problem related to *A. dealbata* is acute in southern Europe since its invaded area is increasing because this species proved to colonize and expand in both disturbed and non-disturbed ecosystems (Hernández *et al.*, 2014; Rodríguez *et al.*, 2017). In particular, *A. dealbata* widely spreads along the western area of the Iberian Peninsula. In NW Spain, the occupied area reached 25,400 ha by 2008 (Hernández *et al.*, 2014) and its invasion is expected to continue spreading mainly in pine forests at a rate of 900 new individuals ha^{-1} yr^{-1} (Rodríguez *et al.*, 2017). In Portugal, although *A. dealbata* has increased its distribution by 400% since the 1970s of the past century (Nunes *et al.*, 2021a) mainly occurring in north and central regions, the quantification of the invaded areas is marginally available (i.e., 6.72 km^2 in Peneda-Gerês National Park, Monteiro *et al.*, 2017). However, a recent model on the *A. dealbata* invasion in NW Spain and north of Portugal predicted an increase of up to 85% of the study area by 2070 under current and future climatic scenarios (Fernandes *et al.*, 2019). A first estimation on *A. dealbata* biomass for an invaded area in central Portugal shows a 133.40 ± 103.66 kg of total weight and 9.42 ± 5.27 kg yr^{-1} of annual productivity on average (Nunes

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et al., 2021b). This study has some limitations because data were obtained from only 11 young trees located in a local area with a diameter at breast height of 8.97 ± 1.66 cm and age ranged from 5 to 20 yr old. However, the values provided by Nunes *et al.* (2021b) will probably increase when considering larger trees. In addition, *A. dealbata* is a fast-growing species that forms large and thick populations that provide a substantial amount of fresh biomass especially by re-sprouting after cutting, fire or frost (Lorenzo *et al.*, 2010a; Souza-Alonso *et al.*, 2013; personal observation). Current management actions to control invasion by acacias, especially those that exclusively depend on public funds, have failed and were insufficient for large areas (Souza-Alonso *et al.*, 2017). Therefore, alternative approaches such as finding potential uses for acacia residues obtained from management actions that revert in benefits to alleviate control costs while prevents spread invasion reveals as an attractive and more sustainable strategy (Souza-Alonso *et al.*, 2017).

Acacia spp., in particular *A. dealbata*, are an important resource for low-input agriculture, firewood, building, medicinal uses, tools, timber and tannins production, as green manures and as fodder for livestock (De Neergaard *et al.*, 2005; Ngorima and Shackleton, 2019). Besides, as a leguminous species, *Acacia* is an interesting agronomic resource because of its ability to fix atmospheric nitrogen. In fact, woody legumes are used worldwide in intercropping and agroforestry systems for overall improving of soil fertility, and especially for providing nitrogen. Nowadays, perennial legumes are considered a valuable resource for environmental conservation (https://www.forevergreen.umn.edu/). Leaves from woody legumes have potential uses as green manure and fodder for an environmentally friendly approach in low-input farming systems (De Neergaard *et al.*, 2005). Indeed, previous reports have shown that litter from *Acacia mangium* and *Acacia auriculiformis* enriched the soil and favored soil fertility (de Taffin *et al.*, 1991). Compost from *Acacia longifolia* residues resulted in a promising substrate for horticultural purposes (Brito *et al.*, 2015b). Specifically, *A. dealbata* increases the content of N, NH_4^+, NO_3^-, C and P in soil (Lorenzo *et al.*, 2010c; González-Muñoz *et al.*, 2012; Lorenzo and Rodríguez-Echeverria, 2012; Lazzaro *et al.*, 2014). The contribution of *A. dealbata* on soil base cations (Na, K, Mg and Ca) is less known, and responses vary with time (Souza-Alonso *et al.*, 2014, 2015). Given the abundance of this species in the Atlantic Iberian coast (MAGRAMA, 2017), green manure could be a profitable use of fresh tissues (Souza-Alonso *et al.*, 2020); though, these tissues have high polyphenol content (De Neergaard *et al.*, 2005), which could limit the release rate of N due to complexation of this nutrient with reactive polyphenols (Palm *et al.*, 2001). Nevertheless, the use of residues from *A. dealbata* as fertilizer has low costs and minor environmental impacts, which are clear advantages for low-input agriculture. Therefore, an efficient approach for controlling *A. dealbata* might consist of using this legume as green manure for crop production.

Another possible benefit of using *A. dealbata* residues is its potential ability for weed control. Allelopathic potential of *A. dealbata* leachates and extracts with natural concentrations has been found in laboratory experiments (Lorenzo *et al.*, 2010b, 2011; Aguilera *et al.*, 2015), though allelopathic effects have not been consistently reported in the field (Lorenzo *et al.*, 2017). Conversely, water-soluble compounds extracted from *A. dealbata* leaves have a remarkable phytotoxic effect regardless of laboratory bioassay conditions (Lorenzo *et al.*, 2016) and green manure from leaves of this species incorporated into an agricultural soil suppressed dicotyledon weeds in low-weed density sites (Souza-Alonso *et al.*, 2020). In addition, methyl cinnamate found in *A. dealbata* flowers showed herbicide potential on the herbicide-resistant weed *Lolium rigidum* Gaudin (Lorenzo *et al.*, 2020). However, the efficiency of the inhibitory effect depends on the conditions, methodologies and weed species evaluated (Kanatas, 2020). For instance, *A. dealbata* leachates collected in NW Spain during the flowering period have strong inhibitory effects on germination and growth of some grass species (Carballeira and Reigosa, 1999). However, its effectiveness was lower and fluctuated over the year and over shorter periods of time on understory plant species (Lorenzo *et al.*, 2010b, 2011).

Maize (*Zea mays* L.) is one of the most important crops for feed and food worldwide and is a model crop in plant breeding (Liu *et al.*, 2020). Global cereal demand in 2020 is estimated at 2.1 billion tons and will, for the first time, show a major shift in favor of maize with demand estimated at 852 million tons (James, 2003). In the Iberian Atlantic coast, where maize is a major summer crop, *A. dealbata* is one of the most important invasive trees and it is urgent to control its spread. As far as we know, no reports have been published to date on the use of *A. dealbata* for fertilization in maize crops. In view of this, the objective of this study was to assess the potential value of *A. dealbata* residues as green manure and as a tool for weed control for maize crops. This could contribute to conduct more sustainable practices by reducing funds allocated to the *A. dealbata* management in invaded areas and synthetic herbicides and fertilizers in conventional agriculture.

Materials and methods

Plant material

Vegetative aerial biomass (leaves and fine branches, up to 1 cm diameter) of *A. dealbata* were collected in a forestry area in Pontevedra, NW Spain (42°23′04″N, 8°39′10″W), in March and October 2017 and December 2018, before each experiment. Plant material was allowed to dry indoors in the dark at room temperature, ground using a standard garden grinder to provide small particles of about 3 cm (hereafter acacia green manure) and stored in the dark at room temperature until use. Nutrient content was analyzed according to standard protocols (http://cactiweb.webs.uvigo.es/Joomla/index.php/gl/). The nutrient content of acacia green manure collected the first time was 47.93% C, 3.49% N, 2.03 g kg dry weight^{-1} (dw) P, 11.33 g kg dw^{-1} K, 7.10 g kg dw^{-1} Ca and 2.26 g kg dw^{-1} Mg.

Pot experiments

*Experiment 1—*A. dealbata *green manure as a source of nutrients*

An initial pot experiment was carried out under greenhouse conditions between 9 and 42 °C ($T_{min.}/T_{max.}$, respectively) to assess whether acacia green manure can be an alternative to the standard inorganic basal dressing in maize cultivation. The experiment was established on April 20th, 2017 in 15-L pots (33 cm diameter) filled with agricultural soil (Ap horizon) collected from an agricultural field located in the Misión Biológica de Galicia (Pontevedra NW Spain). The average soil at the experimental site before conducting the experiments was sandy loam, and its main physico-chemical characteristics were $pH_{H_2O} = 5.5$, 4.6%

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organic matter (OM), 127 ppm available phosphorous and 202 ppm available potassium.

At the beginning of the experiment, two treatments were established: a control treatment with the standard mineral basal dressing for maize cultivation and an acacia treatment with acacia green manure incorporated into the soil as a partial substitute of basal dressing. For control pots, the basal dressing was calculated according to optimum maize requirements and soil characteristics at a dose of 280 kg ha^{-1} Fertitec (20% N, 10% P_2O_5, 5% K_2O), 49.6 kg ha^{-1} Haifa MKP™ (52% P_2O_5, 34% K_2O) and 3055.4 kg ha^{-1} Lithothamne TimacAgro (36% CaO, 2.5% MgO). Top dressing fertilization with 409.76 kg ha^{-1} of Nitramón (20.5% N) was applied when maize plants reached 80–100 cm, 63 days after sowing. Acacia pots were filled with soil mixed with acacia green manure at a dose of 4013.8 kg dw ha^{-1}. This dose was determined according to the N content of acacia green manure, and was equivalent to the N dose applied in control pots. However, as this acacia dose was limited for P and Ca, these nutrients were supplied with Haifa MKP™ (52% P_2O_5, 34% K_2O) and Lithothamne TimacAgro (36% CaO, 2.5% MgO) at doses of 136.9 and 2920.5 kg ha^{-1}, respectively, to be equivalent to the control treatment.

Two maize hybrids were used in order to estimate the effects of fertilization in a hybrid with high (field corn hybrid A619 × A632), or low (sweet corn hybrid V679 × V576) sink ability. Each pot was sown with three seeds of A619 × A632 or V679 × V576. After plants reached the V6 stage, 47 days after sowing, each pot was thinned to one plant per pot. Pots were watered with the same quantity of tap water as needed. Weeds were manually removed and left on the top of the soil to decompose.

The experiment was arranged as a split-plot design with three repetitions. The main plot was the treatment and the subplot was maize hybrid. Each experimental plot consisted of three pots.

During the experiment, the following variables were recorded on each maize plant: plant dw at V6 (on thinned plants), vigor (with a scale from 1 = weak to 9 = vigorous plant) at V6, plant height at V6 and flowering, number of leaves at V6 and flowering, basal fluorescence (F_0) and maximum quantum efficiency of photosystem II (F_v/F_m) at V6, the net photosynthetic rate at V6 and flowering, days to anthesis, days to silking and anthesis–silking interval (ASI). F_0 and F_v/F_m were assessed using an OS-30p Chlorophyll Fluorometer (Opti-Sciences, Inc., Hudson, NH, USA) in the last fully developed leaf. The net photosynthetic rate was recorded with a portable photosynthesis system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) in the ear leaf. Five months after sowing, plant and ear height were measured, maize was harvested and plant and ear dw were determined after drying plant material at 70 °C until constant weight.

At the end of the experiment, soil samples were collected from each pot, air-dried, sieved through a 1 mm mesh and analyzed following standard protocols (http://cactiweb.webs.uvigo.es, Castro-Díez *et al.*, 2012) to determine the contents of N, C, K, P and NO_3^-.

Experiment 2—assessment of temporal nutrient release by acacia green manure

Based on the results from experiment 1, a second experiment was designed in order to understand the temporal pattern of nutrient release in soil, and therefore to determine how long before sowing it is necessary to incorporate acacia green manure into the soil for nutrients to be available for maize growing. In this second experiment, plants were grown in 15-L pots (33 cm diameter) filled with the same agricultural soil (sieved through 2 cm × 1.5 cm mesh) used in the previous experiment and placed outdoors. Acacia green manure was incorporated into these pots into the first 15 cm soil layer at a dose of 9353.4 kg ha^{-1} 6 (November 15th, 2017), 4 (January 15th, 2018) and 2 (March 15th, 2018) months before maize sowing (hereafter, acacia T6, acacia T4 and acacia T2, respectively). This acacia green manure dose contained approximately twice as much N as the dose used in experiment 1 and was thought to prevent N deficiency by leachates because of the rain. P and Ca limitations in acacia green manure were supplemented with Haifa MKP™ (52% P_2O_5, 34% K_2O) and Lithothamne TimacAgro (36% CaO, 2.5% MgO) at doses of 89.0 and 2797.2 kg ha^{-1}, respectively, at maize sowing time. In experiment 1, the acacia green manure dose was calculated as the amount required for providing as much N as a conventional inorganic fertilization, and the P and Ca of the control fertilization was calculated based on the amount contained in the acacia green manure dose. As that fertilization was not enough and/or did not release at an adequate time, the acacia green manure dose was increased for this second experiment. Control treatment was equal for experiments 1 and 2 and consisted of a conventional basal and top dressing with the same mineral fertilizers applied at the same doses as previously described and the top-dressing fertilizer was 27% N (half as nitric and half as ammonium) and 3.5% MgO. Control basal dressing was incorporated into the first 15 cm soil layer at maize sowing. The experiment followed a randomized complete block design with three repetitions, and three pots per experimental plot. Pots received natural rainfall and weeds were periodically removed and left on the soil to decompose.

On May 15th, 2018, pots were sown with three seeds of the highly N demanding maize hybrid A619 × A632. Maize seedlings were thinned to one per pot at V3 stage and left to grow for almost 5 months (until October 11th, 2018). The following variables were recorded throughout the experiment: vigor at V6, aspect at flowering, plant height at V6, number of leaves at V6 and flowering, relative leaf chlorophyll content (SPAD) at V6, flowering and before harvest, F_0 and F_v/F_m at flowering and before harvest, the net photosynthetic rate at flowering, days to anthesis, days to silking, ASI and plant and ear height at harvest. After harvest, the plant and ear leaf dw, presence/absence of grains in the ear, number and dw of grains and of 100-kernels (100k) for each pot were determined. SPAD was recorded with a hand-held CCM-200 Chlorophyll Content Meter (Opti-Sciences, Inc., Hudson, NH, USA). F_0, F_v/F_m, net photosynthetic rate and dws were assessed as described in experiment 1.

On November 11th, 2017, two samples of the agricultural soil were collected by mixing soil from a representative set of the experimental area, and this agricultural soil was mixed with or not with acacia green manure (acacia T0 and control T0) to determine initial values for soil samples, but no maize was sown in these pots. At the end of the experiment, soil samples were collected in each pot, air-dried, sieved (1-mm mesh) and analyzed according to standard protocols (http://cactiweb.webs.uvigo.es, Castro-Díez *et al.*, 2012) to determine the contents of N, C, OM, NO_2^-, NO_3^- and NH_4^+.

Field evaluation

Experiment 3—evaluation of acacia green manure performance under field conditions

Experiment 2 indicated that most of the assessed parameters in maize grown in pots containing acacia green manures for 4 months had similar values compared to control maize plants.

Therefore, this treatment was selected for field evaluation of the potential of acacia green manure for fertilization and weed control in maize cultivation.

The experimental area was established in Pontevedra, NW Spain (42°24′19.6″N, 8°38′33.2″W) characterized by a humid and mild climate with a rainfall of ≈1600 mm and a mean temperature of 14.5°C and sandy-loam agricultural soils (Álvarez-Iglesias, 2015). This area was previously devoted to maize production. Two experimental sites located 350 m apart from each other were selected. In each site, an experimental area covering 195 m^2 was established and split into four 6 m × 5 m plots following a split-plot design with two repetitions. The main plots were the fertilization treatments (acacia green manure at 0.9 kg dw m^{-2}, and conventional fertilization), while the secondary plots were two maize hybrids (C123 × B14A and A632 × W117), in order to allow estimation of the genotype × environment interactions, and had 5 m^2/sub-plot, with 3–10 plant rows separated by 0.8 m between rows and by 0.21 m between plants, given an approximate density of 60,000 plants ha^{-1}.

Acacia and control plots were ploughed and earth-milled on January 15th, 2019. Acacia green manure was immediately applied to acacia plots, left on the soil surface for 1 day and incorporated into the soil by disk harrowing, allowing it to decompose for 4 months. In May 2019, a basal mineral dressing (YaraMila™ ACTYVA: 20% N total, 9.4% nitric acid, 10.6% ammonium, 12.2% P_2O_5, 10% K_2O, 3% MgO, 10% SO_3) were spread on the soil surface of control plots at a dose of 342.9 kg ha^{-1} and immediately rototilled into the soil the day before maize sowing. Acacia plots were rototilled as well. Then, plots were sown with the C123 × B14A or A632 × W117 maize hybrids, having two sub-plots per hybrid within a plot, i.e., four sub-plots per treatment. One experimental site was sown on May 3rd, 2019 (hereafter early sowing) and the other one was on May 22nd, 2019 (hereafter late sowing). The two environments differed not only for the sowing date (19 days from early to late sowing), but also for soil composition (pH = 5.9, 4.3% OM, 88 ppm P, 142 ppm K and 38 ppm Mg in early sowing, *vs* pH = 5.8, 5.6% OM, 105 ppm P, 148 ppm K and 18 ppm Mg in late sowing), height above sea level (55 m in early *vs* 35 m in late sowings) and orientation (east in early *vs* west in late sowing).

Control plots received a top dressing (Nitramón: 27% N total, 13.5% nitric acid, 13.5% ammonium) on July 12th and 18th, 2019, for early and late sowing, respectively. Maize plants in both sites were rainfed throughout the experiment, and harvested on October 31st, 2019. Emerged plants were counted in each plot and divided by the area of the plot for calculating stand at the V3 stage. Then, the number of maize plants per sub-plot, the total plant, ear and stalk dw, ear dw/total dw ratio, number of ears, grain dw and 100k dw were determined as previously described.

Aerial biomass of weeds was sampled in four random frames per plot excluding marginal plot areas to avoid border effects at 3 months after green manure incorporation (T0, on April 29th, 2019), within a month after maize sowing (T1, on May 30th and June 6th, 2019, for early and late sowing, respectively) and at harvest time (T2, on October 31st, 2019). Sampling was conducted in 25 cm × 25 cm frames in the first sampling. However, the sampling size was increased to 50 cm × 50 cm frames. Weeds were cropped at the ground level, classified into three groups (monocotyledons, dicotyledons and *Cyperus* sp. as the major and most problematic weed in both sites) when possible and dried at 70 °C until constant weight. Determinations included biomass of each weed group and total weeds and, for T1, weed density as a number of plants m^{-2}. For T1 in late sowing, neither dicotyledon nor monocotyledon weeds apart from *Cyperus* sp. were found; therefore, only biomass and density of *Cyperus* sp. accounted for total weeds.

Soil samplings were conducted immediately after acacia green manure incorporation (on January 16th), immediately after maize sowing (on April 29th and May 25th, 2019, for early and late sowing, respectively) and at harvest (on October 31st, 2019). Three composed samples were randomly collected from the top 15–20 cm of soil with a hand shovel in each plot at each sampling time. Soil samples were air-dried, sieved through a 1-mm mesh and analyzed to determine the contents of N, C, NO_2^-, NO_3^- and NH_4^+ in soil as previously described.

Statistical analyses

Two-way analyses of variance were conducted *via* general linear models (LMs) for continuous variables or generalized linear models (GLMs) with Poisson error and log link in case of count variables to test for the effect of treatment (acacia green manure and control), maize hybrid (field corn and sweet corn), and the interaction between these two fixed factors on the determined maize and soil variables obtained from experiment 1. If the interaction between treatment and hybrid was significant, a separate analysis for each hybrid was conducted with treatment as a fixed factor.

To explore the effect of treatment (acacia T2, T4 and T6, and control; fixed factor) on determined agronomic traits of maize and soil nutrients from experiment 2, one-way LMs were used for continuous variables and one-way GLMs with Poisson error and log link for count variables. In soil analyses, soil at the beginning of the experiment (acacia and control T0) was included as extra levels in the treatment factor.

Maize data from experiment 3 were initially analyzed bythree-way analyses of variance *via* LMs when variables were continuous and GLMs with Poisson error and log link for count variables. Treatment (acacia green manure and control), hybrid (C123 × B14A and A632 × W117), environment (early and late maize sowing) and interactions among all these factors were considered as fixed factors. The environment × treatment interaction was significant. Therefore, we separately conducted two-way LMs and GLMs for each environment with treatment, hybrid and their interaction as fixed factors. Because weeds and soil were sampled at the plot level regardless of hybrid, the effect of treatment (acacia green manure and control), environment (early and late maize sowing) and the interaction between these two factors on weed and soil variables from experiment 3 were tested using two-way LMs and GLMs with Poisson error and log link for continuous and count variables, respectively.

Post-hoc mean comparisons after all conducted analyses from all experiments were tested pairwise using the least significant difference (LSD) test. The LMs and GLMs were conducted using the 'stats' package, while post-hoc comparisons were performed with the 'agricolae' package, both packages in R, version 3.6.2 (R Development Core Team, 2015). The level of significance was set at $P \leq 0.05$ for all analyses.

Results

Experiment 1

The treatment had a significant effect on plant dw at V6, plant height at V6 and flowering, F_0, F_v/F_m, ASI, plant height and

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Table 1. Mean comparisons of two maize hybrids (field corn and sweet corn) grown in pots with two fertilization treatments (acacia green manure *vs* control) in experiment 1 (2017)

	Treatment			Hybrid		
Response variable	Acacia green manure	Control	LSD	Field corn	Sweet corn	LSD
Plant dw at V6 (kg)	0.020 ± 0.007 b	0.023 ± 0.005 a	NC[a]	0.022 ± 0.007	0.023 ± 0.005	–
Vigor (1–9)[b]	7.25 ± 0.30	8.50 ± 0.12	–	7.89 ± 0.24	7.86 ± 0.32	–
Plant height at V6 (cm)	34.2 ± 1.93 b	39.2 ± 1.49 a	3.93	40.6 ± 1.50 a	31.7 ± 1.43 b	NC
Plant height at flowering (cm)	114 ± 3.98 b	137 ± 4.86 a	NC	114 ± 3.79 b	142 ± 4.31 a	NC
Number of leaves at V6	5.62 ± 0.62	5.62 ± 0.62	–	5.72 ± 0.67	5.5 ± 0.52	–
Number of leaves at flowering	11.6 ± 0.26	12.5 ± 0.24	–	11.8 ± 0.33	12.4 ± 0.15	–
Basal fluorescence (F_0) at V6	78.6 ± 5.06 a	56.6 ± 1.6 b	11.89	68.7 ± 5.10	66.4 ± 3.87	–
Efficiency of photosystem II (F_v/F_m) at V6	702 ± 19.5 b	785 ± 3.05 a	44.07	749 ± 17.60	737 ± 16.60	–
Net photosynthetic rate at V6 (μmol CO_2 m^{-2} s^{-1})	24.6 ± 1.29	26.4 ± 1.97	–	23.4 ± 1.80	28.2 ± 1.22	–
Net photosynthetic rate at flowering (μmol CO_2 m^{-2} s^{-1})	17.9 ± 0.83	19.6 ± 0.63	–	18.6 ± 0.71	19.0 ± 0.82	–
Days to silking	77.8 ± 2.37 a	69.9 ± 1.62 b	NC	80.4 ± 1.43 a	64.5 ± 0.63 b	NC
Days to anthesis	70.8 ± 1.15	67.9 ± 1.23	–	73.2 ± 0.51 a	63.9 ± 0.57 b	NC
ASI (days)	7.0 ± 1.41 a	2.0 ± 0.52 b	NC	7.17 ± 1.18 a	0.62 ± 0.28 b	NC
Plant height (cm)	241 ± 8.15 a	227 ± 6.53 b	NC	256 ± 4.21 a	203 ± 3.68 b	NC
Ear height (cm)	65.2 ± 7.14	76.2 ± 5.42	–	89.6 ± 4.27 a	46.5 ± 3.21	NC
Plant dw (kg)	0.067 ± 0.003 b	0.131 ± 0.005 a	NC	0.109 ± 0.010 a	0.088 ± 0.006 b	NC
Ear dw (kg)	0.021 ± 0.004 b	0.080 ± 0.002 a	–	0.049 ± 0.009	0.052 ± 0.006	–

Mean values ± standard errors are shown.
dw, dry weight.
Means for each variable within each fixed factor and without statistical letters are not significantly different, according to Supplementary Table A.1 and the LSD at $P \leq 0.05$, when followed by different letters statistical differences were found.
aNC means that the LSD cannot be calculated because of the lack of data.
bVigor was estimated by using a scale from 1 = weak plant to 9 = vigorous plant.

plant and ear dw of maize (Supplementary Table A.1). Acacia green manure incorporated into the soil at the same time compared to control fertilization reduced the plant dw at V6, plant height at V6 and flowering, F_v/F_m and plant and ear dws, but increased F_0, days to silking, ASI and plant height (Table 1). Field and sweet corn significantly differed in plant height at V6 and flowering, days to silking, days to anthesis, ASI, height and weight of plant and ear (Supplementary Table A.1), with field corn showing higher values for all of these variables, except for plant height at V6 (Table 1). The interaction between treatment and hybrid was significant for dw and height of the plant at V6 and plant and ear dws (Supplementary Table A.1). Analyzing each hybrid separately, field corn in control pots had a higher plant dw at V6 and plant and ear dws than that in pots containing acacia green manure (Supplementary Table A.2, Table 2). Sweet corn showed increased height at V6 and plant and ear dws in control pots (Supplementary Table A.2, Table 2). Conversely, plant dw at V6 was not significantly different between acacia and control pots for sweet corn.

After growing maize, soil collected in pots with acacia green manure had the same content of N, C, K and NO_3 than that in control pots, but acacia pots were deficient in P (Supplementary Table A.3, Table 3). The type of hybrid had a significant effect on C and NO_3^- soil content (Supplementary Table A.3). Pots without maize showed a higher value of C compared to pots with sweet corn (Table 3). The presence of maize in pots drastically reduced the content of NO_3^- in soil (Table 3). The interaction between treatment and hybrid did not affect soil nutrients (Supplementary Table A.3).

Experiment 2

The incorporation of acacia green manure at different times before maize sowing had a significant effect on the chlorophyll content at V6 and flowering, plant dw and number of grains. However, control and acacia treatments showed non-significant differences for most physiological and agronomic traits (Supplementary Table A.4). Maize grown in acacia T4 and T6 pots showed the same chlorophyll content at V6 and flowering and plant dw, but higher dw of grains compared to maize from control treatment (Table 4). However, acacia T2 reduced the chlorophyll content at V6 and flowering and plant dw compared to control (Table 4).

Regarding soil nutrients, the content of NH_4^+ was significantly affected by treatment, being the highest at acacia T0 (without maize) and the lowest at pots after growing maize in acacia T4 soils, although differences were only significant between acacia T0 and T4 (Supplementary Table A.5, Table 5). The contents of

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Table 2. Mean comparisons among treatment × hybrid interactions of two maize hybrids grown in pots with two fertilization treatments (acacia green manure *vs* control) in experiment 1 (2017)

Response variable	Treatment × hybrid interaction					
	Acacia green manure field corn	Control field corn	LSD	Acacia green manure sweet corn	Control sweet corn	LSD
Plant dw at V6 (kg)	0.019 ± 0.004 b B	0.024 ± 0.007 a A	NC[a]	0.025	0.023 ± 0.008	–
Vigor (1–9)[b]	7.4 ± 0.41	8.3 ± 0.17	–	7.0 ± 0.47	8.7 ± 0.16	–
Plant height at V6 (cm)	40.1 ± 1.75 a	41.2 ± 2.52 a	–	26.8 ± 1.27 b B	36.6 ± 1.03 a A	4.04
Plant height at flowering (cm)	105 ± 4.83	124 ± 4.02	–	128 ± 2.75	154 ± 5.27	–
Number of leaves at V6	5.78 ± 0.22	5.67 ± 0.24	–	5.43 ± 0.18	5.57 ± 0.18	–
Number of leaves at flowering	11.2 ± 0.43	12.4 ± 0.41	–	12.2 ± 0.14	12.6 ± 0.26	–
Basal fluorescence (F_0) at V6	79.9 ± 8.51	57.4 ± 2.58	–	77.3 ± 5.56	55.6 ± 1.9	–
Efficiency of photosystem II (F_v/F_m) at V6	708 ± 30.1	789 ± 3.09	–	693 ± 26.0	780 ± 5.22	–
Net photosynthetic rate at V6 (μmol CO_2 m^{-2} s^{-1})	23.9 ± 1.85	22.8 ± 3.21	–	25.4 ± 1.89	31.0 ± 0.93	–
Net photosynthetic rate at flowering (μmol CO_2 m^{-2} s^{-1})	17.3 ± 0.76	20.0 ± 1.06	–	18.9 ± 1.65	19.0 ± 0.66	–
Days to silking	85.1 ± 1.69	75.7 ± 0.47	–	66.8 ± 0.39	62.6 ± 0.60	–
Days to anthesis	74.3 ± 0.62	72.1 ± 0.63	–	65.5 ± 0.72	62.6 ± 0.60	–
ASI (days)	10.8 ± 1.53	3.56 ± 0.58	–	1.33 ± 0.50	0.0 ± 0.0	–
Plant height (cm)	226 ± 6.16	247 ± 3.88	–	205 ± 4.28	201 ± 6.14	–
Ear height (cm)	86.8 ± 7.4	92.4 ± 4.58	–	37.6 ± 2.28	55.4 ± 4.35	–
Plant dw (kg)	0.071 ± 0.005 c B	0.148 ± 0.004 a A	12.56	0.061 ± 0.003 c X	0.110 ± 0.003 b Y	NC
Ear dw (kg)	0.014 ± 0.004 c B	0.084 ± 0.002 a A	9.64	0.030 ± 0.005 b X	0.075 ± 0.001 a Y	12.48

Mean values ± standard errors are shown.
dw, dry weight.
Means for each variable and without statistical letters are not significantly different, according to Supplementary Table A.1 and LSD test at $P \leq 0.05$, when followed by different lowercase letters statistical differences were found. Different capital letters refer to significant differences between treatments for each hybrid according to significant *P* values from Supplementary Table A.2 and LSD test at $P \leq 0.05$.
aNC means that the LSD cannot be calculated because of the lack of data.
bVigor was estimated by using a scale from 1 = weak plant to 9 = vigorous plant.

N, C, OM, NO_2^- and NO_3^- in soil were not affected by treatment (Supplementary Table A.5, Table 5).

Experiment 3

The application of acacia green manure in the field significantly reduced the total weed biomass at T0, biomass and density of dicotyledons, *Cyperus* sp. and total weeds and density of monocotyledons at T1, and biomass of monocotyledons, dicotyledons and total weeds at T2 (Supplementary Table A.6, Table 6). Specifically, the biomass reduction in total weeds achieved a 32, 46 and 44% in T0, T1 and T2, respectively. The environment factor significantly influenced the biomass of total weeds at all sampling times (Supplementary Table A.6). This factor also affected the density of *Cyperus* sp. and total weeds at T1 and biomass of dicotyledons at T2 (Supplementary Table A.6). Early sowing favored the biomass of total weeds regardless of sampling time, density of total weeds at T1 and biomass of dicotyledons at T2, while reduced the number of individuals of *Cyperus* sp. at T1 (Table 6). The interaction between treatment × environment was significant for the number of *Cyperus* sp. and total weeds at T1 and biomass of monocotyledons and total weeds at T2 (Supplementary Table A.6). In the early sowing, green acacia manure always reduced the weed parameters significantly affected by the interaction (Supplementary Table A.7, Table 7). However, in late sowing, acacia treatment reduced the number of total weeds (*Cyperus* sp.) at T1, but it did not affect the biomass of monocotyledons and total weeds at T2 (Supplementary Table A.7, Table 7).

The three-way analysis of variance conducted on maize data found significant effects for plant and stalk dw by treatment, and for plant, ear and stalk dw, ear dw/total dw ratio, number of ears, grain dw and 100k dw by the environment (Supplementary Table A.8). This analysis also found a significant effect of the treatment × environment interaction on the ear dw/total dw ratio (Supplementary Table A.8). Maize growing in plots with acacia green manure had 45% lower plant and stalk dw compared to those in control plots (Table 8). In general, maize plants in early sowing were much smaller than those in late sowing (Table 8). According to the treatment × environment interaction, the ratio ear dw/total dw was higher for maize in control plots at late sowing, but it was the same for control and acacia green manure treatments at early sowing (Table 8). Because of the treatment × environment interaction was significant, a two-way analyses of variance was conducted separately

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Table 3. Mean comparisons of soil from pot where two maize hybrids were grown with two fertilization treatments (acacia green manure *vs* control) in experiment 1 (2017)

Fixed factor	Category	N (%)	C (%)	K (mg kg^{-1})	P (mg kg^{-1})	NO_3^- (mg kg^{-1})
Treatment (T)	Acacia green manure	0.23 ± 0.009	3.61 ± 0.038	2594 ± 88.5	1471 ± 13.0 a	7.89 ± 3.74
	Control	0.22 ± 0.002	3.67 ± 0.034	2579 ± 49.3	1573 ± 25.5 b	11.00 ± 5.39
	LSD	–	–	–	64.32	–
Hybrid (H)	Field corn	0.23 ± 0.010	3.63 ± 0.031 ab	2620 ± 122.0	1495 ± 27.8	3.64 ± 0.92 b
	Sweet corn	0.22 ± 0.007	3.58 ± 0.040 b	2534 ± 22.7	1534 ± 25.5	2.44 ± 0.37 b
	Without maize	0.23 ± 0.003	3.75 ± 0.032 a	2614 ± 85.7	1546 ± 55.8	28.7 ± 6.09 a
	LSD	–	NC[a]	–	–	NC
T × H	Acacia green manure field corn	0.24 ± 0.020	3.63 ± 0.047	2722 ± 241.0	1458 ± 16.5	1.66 ± 0.50
	Control field corn	0.22 ± 0.003	3.63 ± 0.052	2518 ± 74.7	1532 ± 46.9	5.62 ± 0.24
	Acacia green manure sweet corn	0.22 ± 0.015	3.52 ± 0.038	2518 ± 28.7	1490 ± 29.1	2.99 ± 0.48
	Control sweet corn	0.23 ± 0.003	3.64 ± 0.055	2550 ± 38.7	1579 ± 19.8	1.89 ± 0.39
	Acacia green manure without maize	0.23 ± 0.000	3.64 ± 0.055	2516 ± 51.2	1465 ± 23.2	24.60 ± 4.03
	Control without maize	0.22 ± 0.000	3.78 ± 0.025	2713 ± 148.0	1627 ± 70.6	32.80 ± 13.10

Mean values ± standard errors are shown.
Means for each variable and each fixed factor combination without statistical letters are not significantly different, according to Supplementary Table A.3 and the LSD test at $P \leq 0.05$, when followed by different letters statistical differences were found.
aNC means that the LSD cannot be calculated because of the lack of data.

for each environment. We neither found significant effects by treatment or hybrid, nor by the interaction between these two factors in early sowing (Supplementary Table A.9, Table 9). For late sowing, significant effects of treatment were only found on the ear dw/total dw ratio and dw of 100 grains (Supplementary Table A.9). Acacia green manure reduced the ear dw/total dw ratio of maize plants but also increased the 100k dw (Table 9). No significant effects were found for any of the other parameters evaluated (Supplementary Table A.9, Table 9).

Treatment had a significant effect on N, C and NO_3^- at incorporation time of acacia green manure (T0), on NO_3^- and NH_4^+ at the application of conventional fertilization (T1) and on NO_2^- at harvest time and end of the experiment (T2) (Supplementary Table A.10). At T0, the contents of N, C and NO_3^- were higher in soils with acacia green manure than those in control soils. However, their content rapidly increased (NO_3^- and NH_4^+) in soils recently treated with conventional fertilization (T1) and the content of NO_2^- was still higher in the control treatment at harvest time (T2) (Table 10). On the other hand, the environment factor significantly affected N, C, NO_2^-, NO_3^- and NH_4^+ at T0, N, C, NO_3^- and NH_4^+ at T1, and N, C and NO_2^- at T2 (Supplementary Table A.10). Soil collected from the early sowing had a lower contents of N and C at T0, T1 and T2, NO_2^- at T0 and T2 and a higher content of NO_3^- and NH_4^+ at T0 and T1 (Table 10). The contents of NO_3^- at T0 and NO_3^- and NH_4^+ at T1 were additionally affected by the treatment × environment interaction (Supplementary Table A.10). This effect varied depending on sowing date (Supplementary Table A.11). In the early sowing, the content of NO_3^- at T0 was higher in soils with acacia green manure than that in control (Supplementary Table A.11, Table 10). However, control soil showed higher NO_3^- and NH_4^+ at T1 (Supplementary Table A.11, Table 10). In late sowing, significant differences were only found for NO_3^- at T1, which was higher in the control treatment (Supplementary Table A.11, Table 10).

Discussion

Experiment 1 showed that acacia green manure incorporated at sowing time reduced maize biomass at all stages, and plant height at early stages of development, along with the efficiency of photosystem II, while increased F_0, time to flowering and ASI. These results indicate that fertilizing with acacia at sowing time produced low-nutrition stress. The reason for this malnutrition is that acacia requires a period of decomposition in order to release nutrients that could be used by maize (Castro-Díez *et al.*, 2012). Similarly, Gutteridge (1992) evaluated leaf mulch of the *Acacia cunninghamii* and *A. fimbriata*, among other tree legumes, as a source of nitrogen for maize growth in greenhouse pot experiments, and concluded that these two acacia species were ineffective as sources of nitrogen in the short term. They hypothesize that the poor response to acacia mulch may be due to the high polyphenol and lignin content of the leaf. Similarly, *A. dealbata* leaves predominantly contain resorcinol, maculosin and moretenone (Aguilera *et al.*, 2015) and other terpenic compounds (Oliveira *et al.*, 2020). Castro-Díez *et al.* (2012) explained that, despite *A. dealbata* produces a high amount of nutrient-rich litter that decomposes and releases nutrients into the soil, the presence of secondary metabolites counteract that effect.

As expected, the field corn hybrid had significantly larger plants at V6 and flowering, days to silking, days to anthesis, ASI, plant height and plant and ear dw than the sweet corn hybrid; because the field corn hybrid had larger biomass and yield potential compared to the sweet corn hybrid. However, sweet corn has lower nutrient requirements compared to field corn grain (Treat and Tracy, 1994). Accordingly, the highest sink effect of field corn caused more important differences between control and acacia green manure than for the sweet corn hybrid, which has the lowest nutrient requirements. Finally, the soil analyses revealed that greenhouse conditions and the limited pot size reduced the ability of maize for

Table 4. Mean comparisons of maize grown in pots with four fertilization treatments (acacia green manure T6, T4 and T2[a] and control) in experiment 2 (2018)

	Treatment				
Response variable	Acacia T2	Acacia T4	Acacia T6	Control	LSD
Vigor at V6 stage (1–9)[b]	4.44 ± 0.48	6.00 ± 0.54	4.83 ± 0.79	5.67 ± 0.67	–
Aspect at flowering (1–9)[b]	4.25 ± 0.43	5.43 ± 0.30	4.83 ± 0.48	5.75 ± 0.24	–
Plant height at V6 stage (cm)	21.75 ± 1.64	27.6 ± 2.03	24.2 ± 2.61	27.2 ± 2.48	–
Number of leaves at V6 stage	2.56 ± 0.18	3.14 ± 0.14	2.67 ± 0.21	3.22 ± 0.22	–
Number of leaves at flowering	4.50 ± 0.36	5.71 ± 0.29	4.83 ± 0.40	5.50 ± 0.18	–
Chlorophyll SPAD at V6 stage	7.47 ± 0.65 b	7.76 ± 0.22 ab	9.20 ± 1.05 ab	10.60 ± 1.01 a	NC[c]
Chlorophyll SPAD at flowering	8.76 ± 1.64 b	10.30 ± 2.38 ab	10.70 ± 2.19 ab	19.80 ± 3.26 a	NC
Chlorophyll SPAD before harvest	9.55 ± 2.26	11.00 ± 4.21	8.50 ± 2.58	13.10 ± 3.54	–
Basal fluorescence (F_0) at flowering	47.20 ± 2.12	51.60 ± 1.36	51.00 ± 2.19	52.40 ± 2.81	–
Efficiency of photosystem II (F_v/F_m) at flowering	0.763 ± 0.005	0.773 ± 0.006	0.750 ± 0.016	0.772 ± 0.011	–
Basal fluorescence (F_0) before harvest	41.20 ± 5.69	40.10 ± 6.88	42.00 ± 8.64	57.00 ± 2.83	–
Efficiency of photosystem II (F_v/F_m) before harvest	0.636 ± 0.087	0.622 ± 0.104	0.589 ± 0.119	0.702 ± 0.025	–
Photosynthetic rate at flowering (µmol CO_2 m^{-2} s^{-1})	18.60 ± 1.36	20.30 ± 2.84	17.70 ± 2.77	18.80 ± 2.12	–
Days to silking	91.6 ± 2.12	86.0 ± 1.45	91.0 ± 2.61	85.5 ± 1.70	–
Days to anthesis	85.6 ± 1.67	81.1 ± 0.91	86.3 ± 2.53	84.0 ± 2.54	–
ASI (days)	6.00 ± 1.26	4.86 ± 1.16	4.67 ± 1.17	3.38 ± 0.67	–
Plant height (cm)	153 ± 8.15	166 ± 5.48	165 ± 8.10	170 ± 5.37	–
Ear height (cm)	32.5 ± 4.00	36.9 ± 2.36	47.8 ± 6.59	39.6 ± 3.14	–
Plant dw (kg)	0.031 ± 0.002 b	0.040 ± 0.004 ab	0.038 ± 0.004 ab	0.049 ± 0.005 a	NC
Ear dw (kg)	0.028 ± 0.007	0.059 ± 0.009	0.044 ± 0.011	0.066 ± 0.014	–
Grains in ear (yes/no)	0.75 ± 0.15	1.00 ± 0.00	0.83 ± 0.17	0.89 ± 0.11	–
dw of grains (g)	21.6 ± 2.48	28.2 ± 1.21	25.6 ± 1.88	25.9 ± 2.95	–
Number of grains	88.6 ± 7.83 b	98.6 ± 1.43 a	100 ± 0.00 a	89.7 ± 8.32 b	NC
dw of 100k (g)	24.8 ± 1.69	28.6 ± 1.24	25.6 ± 1.88	29.3 ± 2.12	–

Mean values ± standard error are shown.
dw, dry weight.
Means for each variable and without statistical letters are not significantly different, according to Supplementary Table A.4 and the LSD test at $P \leq 0.05$, when follow by different letters statistical differences were found.
aT6, T4 and T2: acacia green manure incorporated into the soil 6, 4 and 2 months before maize sowing.
bVigor and aspect were estimated by using a scale from 1 = weak plant to 9 = vigorous plant.
cNC means that the LSD cannot be calculated because of the lack of data.

removing of nutrients and, therefore, the detection of significant effects.

Consequently, experiment 2 was performed in pots outside the greenhouse, with the field corn hybrid alone and incorporating acacia green manure in advance, at different dates, in order to find out the period required for releasing nutrients by acacia leaves. However, control and acacia fertilization had non-significant differences in most physiological and agronomic traits. Merely, acacia green manures incorporated 4 and 6 months before maize sowing showed higher dw of grains compared to maize with control fertilization. Conversely, incorporating acacia 2 months before maize sowing reduced the chlorophyll content at V6 and flowering and the plant dw compared to control. The values for most of the physiological and agronomic parameters of maize in acacia T4 were more close to those in the control fertilization compared to in acacia T6. Therefore, 4 months was the period required for releasing nutrients by acacia leaves. Soil analyses also supported this conclusion. A 4 month period for releasing nutrients fromacacia leaves in enough quantity for maize growth found in our study was faster than that indicated for the decomposition of *A. dealbata* litter (Castro-Diez *et al.*, 2012). These authors found that nitrate released by litter appears in the soil after 4 months, being highest after the 9th month. In a similar experiment, Partey *et al.* (2018) evaluated the nitrogen supplying capabilities of ten leaf biomass sources, including *A. auriculiformis*, for maize production and concluded that most plant residues increased nitrogen concentration and reduced the C/N ratio without effects on nitrogen mineralization patterns. They also found that the nitrogen release from leaf biomass of *A. auriculiformis* takes 14 days and that all green manures tested increased yield and N uptake by maize being comparable with inorganic fertilizer (Partey *et al.*, 2018). Residues from other acacias, such as *A. longifolia* and *A. melanoxylon* need to be composted for an extended period to release nutrients (Brito *et al.*,

Table 5. Mean comparisons of soil from pots without maize (acacia green manure T0[a] and control T0) and soil from pots where maize was grown with four fertilization treatments (acacia green manure T6, T4 and T2[b] and control) in experiment 2 (2018)

Treatment	N (%)	C (%)	OM (%)	NO_2^- (mg kg^{-1})	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})
Acacia T0	0.24 ± 0.010	2.64 ± 0.070	4.55 ± 0.121	0.17 ± 0.011	7.28 ± 2.09	11.80 ± 0.58 a
Control T0	0.24 ± 0.005	2.60 ± 0.025	4.47 ± 0.043	0.20 ± 0.021	12.80 ± 1.06	9.88 ± 0.32 ab
Acacia T2	0.24 ± 0.005	2.52 ± 0.049	4.34 ± 0.084	0.15 ± 0.025	9.36 ± 2.37	7.22 ± 0.91 ab
Acacia T4	0.23 ± 0.003	2.54 ± 0.043	4.37 ± 0.073	0.21 ± 0.017	13.50 ± 2.44	4.36 ± 0.91 b
Acacia T6	0.24 ± 0.004	2.57 ± 0.044	4.42 ± 0.076	0.16 ± 0.019	10.80 ± 1.54	6.95 ± 1.45 ab
Control	0.24 ± 0.003	2.60 ± 0.036	4.48 ± 0.062	0.16 ± 0.023	7.66 ± 2.20	7.05 ± 1.19 ab
LSD	–	–	–	–	–	NC[c]

Mean values ± standard error are shown.
OM, organic matter.
Means for each variable and fixed factor combination and without statistical letters are not significantly different, according to Supplementary Table A.5 and the LSD test at $P \leq 0.05$, when followed by different letters statistical differences were found.
aT0: soil at the beginning of the experiment with or without acacia green manure immediately incorporated, both without sowing maize.
bT6, T4 and T2: acacia green manure incorporated into the soil 6, 4 and 2 months before maize sowing.
cNC means that the LSD cannot be calculated because of the lack of data.

Table 6. Mean comparisons of weeds grown in field maize plots with two fertilization treatments (acacia green manure incorporated into the soil 4 months before sowing *vs* control) in two environments in experiment 3 (2019)

	Treatment			Environment		
Response variable	Acacia green manure	Control	LSD	Early sowing	Late sowing	LSD
Biomass of total weeds at T0 (g m^{-2})	143 ± 17.6 b	211 ± 28.4 a	64.06	215 ± 23.6 a	139 ± 19.1 b	64.06
Biomass of monocotyledon weeds at T1 (g m^{-2})	51.7 ± 11.2	52.4 ± 15.9	–	–	–	–
Biomass of dicotyledon weeds at T1 (g m^{-2})	35.3 ± 6.66 b	89.4 ± 18.1 a	41.43	–	–	–
Biomass of *Cyperus* sp. at T1 (g m^{-2})	10 ± 1.59 b	28 ± 3.91 a	NC[a]	18.80 ± 4.14	20.00 ± 3.37	–
Biomass of total weeds at T1 (g m^{-2})	59 ± 13.3 b	109 ± 21.9 a	34.10	132 ± 16.6 a	20 ± 3.37 b	NC
Number of monocotyledon weeds ant T1 (individuals m^{-2})	1412 ± 128 a	760 ± 221 b	18.22	–	–	–
Number of dicotyledon weeds at T1 (individuals m^{-2})	726 ± 160 b	1784 ± 202 a	15.75	–	–	–
Number of *Cyperus* sp. at T1 (individuals m^{-2})	127 ± 30.1 b	210 ± 36.5 a	4.69	78 ± 16.7 b	290 ± 28 a	NC
Number total weeds at T1 (individuals m^{-2})	1349 ± 267 b	1664 ± 320 a	7.55	2419 ± 151 a	290 ± 28 b	NC
Biomass of monocotyledon weeds at T2 (g m^{-2})	432 ± 34.2 b	618 ± 42.6 a	102.38	522 ± 58.6	528 ± 26.2	–
Biomass of dicotyledon weeds at T2 (g m^{-2})	42.3 ± 7.59 b	206 ± 66.9 a	NC	194 ± 62.1 a	58 ± 34.9 b	NC
Biomass of total weeds at T2 (g m^{-2})	464 ± 32.6 b	823 ± 68.4 a	115.64	716 ± 85.2 a	571 ± 45.4 b	115.64

Mean values ± standard errors are shown.
Means for each variable within each fixed factor and without statistical letters are not significantly different, according to Supplementary Table A.6 and the LSD test at $P \leq 0.05$, when followed by different letters statistical differences were found.
T0, incorporation of acacia green manure to acacia plots 4 months before sowing maize. T1, application of conventional fertilization to control plots and maize sowing. T2, harvesting maize and end of the experiment.
aNC means that the LSD cannot be calculated because of the lack of data.

2015a, 2015b). However, results obtained in the experiment 2 suggest that the incorporation of leaves and small branches of *A. dealbata* into the soil accelerates their decomposition and a composting process does not seem to be necessary to release nutrients, which facilitates the residues management.

Based on previous results, experiment 3 was carried out in the field by adding acacia 4 months before maize sowing. In the field, both fertilization and phytotoxic effects could be checked under real conditions. In general, acacia green manure reduced density and biomass of weeds, mainly dicotyledon species, during the entire experiment. At T0, the reduced weed biomass in acacia plots can be explained by the presence of phytotoxic compounds in soil because these plots had a higher content of soil nutrients compared to control plots. However, at T1 (when control plots received inorganic fertilization) and T2, acacia plots showed reduced weed biomass and soil nutrients. In this case, phytotoxicity from leaf residues is likely to continue to occur (Reigosa and Carballeira, 2017), but a negative effect by nutrient limitations cannot be excluded. Despite this, our results support the *in vitro* allelopathic effects previously reported by Lorenzo *et al.*

Table 7. Mean comparisons among treatment × sowing interactions of weeds grown in field maize plots with two fertilization treatments (acacia green manure incorporated into the soil 4 months before sowing *vs* control) in two environments in experiment 3 (2019)

Response variable	Treatment × environment interaction					
	Acacia green manure early sowing	Control early sowing	LSD	Acacia green manure late sowing	Control late sowing	LSD
Biomass of total weeds at T0 ($g\ m^{-2}$)	174 ± 28.2	256 ± 42	–	112 ± 15.8	166 ± 33.2	–
Biomass of monocotyledon weeds at T1 ($g\ m^{-2}$)	51.7 ± 11.2	52.4 ± 15.9	–	–	–	–
Biomass of dicotyledon weeds at T1 ($g\ m^{-2}$)	35.3 ± 6.66 b	89.4 ± 18.1 a	41.43	–	–	–
Biomass of *Cyperus* sp. at T1 ($g\ m^{-2}$)	9.94 ± 2.72	26.6 ± 6.61	–	10.1 ± 1.8	29.8 ± 4.19	–
Biomass of total weeds at T1 ($g\ m^{-2}$)	95.7 ± 14.2	168 ± 24.5	–	10.1 ± 1.8	29.8 ± 4.19	–
Number of monocotyledon weeds ant T1 (individuals m^{-2})	1412 ± 128 b	760 ± 221 a	18.22	–	–	–
Number of dicotyledon weeds at T1 (individuals m^{-2})	726 ± 160 b	1784 ± 202 a	15.75	–	–	–
Number of *Cyperus* sp. at T1 (individuals m^{-2})	42 ± 10 d B	114 ± 26.9 c A	6.38	241 ± 33.9 b X	338 ± 33.9 a Y	7.84
Number total weeds at T1 (individuals m^{-2})	2180 ± 182 d B	2658 ± 220 c A	12.41	241 ± 33.9 b X	338 ± 39.9 a Y	7.84
Biomass of monocotyledon weeds at T2 ($g\ m^{-2}$)	360 ± 40 b B	685 ± 74.8 a A	181.95	505 ± 43.9 ab	550 ± 29.5 ab	113.42
Biomass of dicotyledon weeds at T2 ($g\ m^{-2}$)	52.5 ± 10.6	335 ± 104	–	22 ± 7.35	76 ± 60.6	–
Biomass of total weeds at T2 ($g\ m^{-2}$)	412 ± 44.1 b B	1020 ± 53.2 a A	184.14	516 ± 42.9 b	626 ± 78.3 b	191.55

Mean values ± standard errors are shown.
Means for each variable and without statistical letters are not significantly different, according to Supplementary Table A.7 and LSD test at $P \leq 0.05$, when followed by different lowercase letters, statistical differences were found. Different capital letters refer to significant differences between treatments for each maize sowing according to significant P values from Supplementary Table A.7 and LSD test at $P = 0.05$.
T0, incorporation of acacia green manure to acacia plots 4 months before sowing maize. T1, application of conventional fertilization to control plots and maize sowing. T2, harvesting maize and end of experiment.

(2010b, 2011, 2016) and provide new evidence for herbicide potential in reducing dicotyledon weeds (Souza-Alonso *et al.*, 2020). Weeds also varied depending on the trial. The treatment × environment interactions suggest that the variable effect of plant residues on weed control highly depend on environmental factors, such as site, climatic or soil parameters (Puig *et al.*, 2019; Souza-Alonso *et al.*, 2020).

In the late-field trial, acacia green manure reduced the ear dw/total dw ratio of maize plants but also increased grain dw. At the first sampling, soil from acacia plots had a higher content of N, C and NO_3; though, at subsequent samplings, nutrient content increased with conventional fertilization. This agrees with previous results showing that *Acacia*, particularly *A. dealbata*, enriched the content of nutrients in soil contributing to maintaining the soil fertility (de Taffin *et al.*, 1991; Lorenzo *et al.*, 2010c; González-Muñoz *et al.*, 2012; Lorenzo and Rodríguez-Echeverría, 2012), suggesting that acacia green manures can partially replace conventional fertilization. The treatment × environment interaction affected NO_3^- at T0 and NO_3^- and NH_4^+ at T1, being NO_3^- content higher at T0 with acacia green manure; though control soils had higher NO_3^- and NH_4^+ at T1. Partey *et al.* (2018) concluded that in locations where inorganic fertilizers are limited, fertilization with leaf plant residues provided comparable results in total grain yield of maize to inorganic fertilization, being a plausible alternative for this crop. Similarly, Tomar *et al.* (2013) studied the effect of green leaves of *A. auriculiformis*, *Alnus nepalensis* and other trees in rice and found that yield and nutrient balance was lower than with inorganic fertilizer the first year. However, the third year, green leaf manuring surpassed the control. They concluded that fertilization with plant residues could have long-term implications and maintain soil OM and improve crop yield (Tomar *et al.*, 2013).

Conclusions

Altogether, some of the deficiencies caused by acacia green manure fertilization on maize crop depend on decomposition time, genotype and environment. Fertilization with acacia green manure 4 months before sowing maize could partially replace inorganic fertilization. However, minor deficiencies should be corrected with additional N fertilization, depending on the nutrient demand of the crop and the environment. A previous composting process does not seem to be necessary to release nutrients, which facilitates the *A. dealbata* residues management. In addition, acacia green manure can be used as a complementary management tool to control weeds for 3 months after green manure incorporation, contributing to reduce the use of synthetic herbicides in maize-based cropping systems. The issue of managing invasive species is rare and fraught with biological, social and economic challenges as well as posing difficulties in decision-making for land managers. Traditional management actions have failed to control *A. dealbata* because of its fast-growing and sprouting ability. New policies oriented to facilitate a widespread use of *A. dealbata* residues to agricultural purposes could confine invasive populations and further limit the expansion of acacia to adjacent areas. In addition, communication and collaboration between researchers and decision-makers is key to translate existing research results into relevant policy directions and to identify specific policy needs to drive future research directions.

Table 8. Mean comparisons of two hybrids of maize grown in field plots with two fertilization treatments (acacia green manure incorporated into the soil 4 months before sowing *vs* control) in two environments in experiment 3 (2019)

Fixed effects	Category	Number of plants (sub-plot)	Total plant dw (kg)	Ear dw (kg)	Stalk dw (kg)	Ear dw/total dw ratio	Number of ears (plot)	Grain dw (g)	100k dw (g)
Treatment	Acacia green manure	4.5 ± 0.3	0.149 ± 0.04 b	0.071 ± 0.030	0.032 ± 0.008 b	0.255 ± 0.064	1.88 ± 0.49	56.0 ± 24.10	15.1 ± 3.52
	Control	4.6 ± 0.3	0.271 ± 0.07 a	0.153 ± 0.049	0.058 ± 0.014 a	0.330 ± 0.080	2.31 ± 0.59	119 ± 40.50	12.8 ± 2.99
	LSD	–	0.117	–	0.025	–	–	–	
Environment	Early sowing	4.1 ± 0.4	0.048 ± 0.01 b	0.002 ± 0.001 b	0.012 ± 0.002 b	0.013 ± 0.009 b	0.13 ± 0.09 b	1.2 ± 0.89 b	3.9 ± 2.68 b
	Late sowing	5.0 ± 0.0	0.372 ± 0.06 a	0.222 ± 0.044 a	0.074 ± 0.012 a	0.537 ± 0.037 a	4.06 ± 0.27 a	173.0 ± 36.90 a	24.1 ± 0.78 a
	LSD	–	0.117	0.087	NC[a]	NC	0.433	73.549	5.892
E × T	EA	4.0 ± 0.6	0.038 ± 0.01	0.002 ± 0.002	0.009 ± 0.002	0.019 ± 0.017 c	0.13 ± 0.13	1.7 ± 1.65	4.5 ± 4.51
	EC	4.1 ± 0.6	0.058 ± 0.02	0.001 ± 0.001	0.012 ± 0.004	0.008 ± 0.007 c	0.13 ± 0.13	0.8 ± 0.81	3.2 ± 3.22
	LA	5.0 ± 0.0	0.260 ± 0.06	0.140 ± 0.050	0.052 ± 0.012	0.462 ± 0.056 b	3.62 ± 0.38	110.0 ± 40.50	25.8 ± 0.69
	LC	5.0 ± 0.0	0.483 ± 0.09	0.304 ± 0.061	0.097 ± 0.018	0.613 ± 0.034 a	4.50 ± 0.33	236.0 ± 55.40	22.5 ± 1.15
E × T × H	EA H1	3.8 ± 1.3	0.049 ± 0.02	0.004 ± 0.004	0.013 ± 0.005	0.044 ± 0.038	0.3 ± 0.25	3.3 ± 3.30	9.0 ± 9.02
	EC H1	4.5 ± 0.5	0.051 ± 0.02	0.000 ± 0.000	0.011 ± 0.003	0.000 ± 0.000	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	LA H1	5.0 ± 0.0	0.278 ± 0.06	0.129 ± 0.050	0.056 ± 0.013	0.416 ± 0.080	4.0 ± 0.41	98.0 ± 44.50	25.4 ± 1.29
	LC H1	5.0 ± 0.0	0.565 ± 0.12	0.351 ± 0. 096	0.113 ± 0.023	0.593 ± 0.061	5.0 ± 0.00	285.0 ± 83.50	23.7 ± 1.67
	EA H2	4.3 ± 0.5	0.028 ± 0.00	0.000 ± 0.000	0.007 ± 0.001	0.000 ± 0.000	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	EC H2	3.8 ± 1.3	0.065 ± 0.04	0.003 ± 0.003	0.017 ± 0.008	0.019 ± 0.016	0.3 ± 0.25	1.6 ± 1.61	6.5 ± 6.45
	LA H2	5.0 ± 0.0	0.242 ± 0.12	0.151 ± 0.096	0.049 ± 0.023	0.508 ± 0.082	3.3 ± 0.63	123.0 ± 74.70	26.1 ± 0.68
	LC H2	5.0 ± 0.0	0.401 ± 0.13	0.258 ± 0.087	0.080 ± 0.027	0.633 ± 0.036	4.0 ± 0.58	188.0 ± 76.00	21.2 ± 1.56

Mean values ± standard errors are shown.

dw, dry weight. T, treatment; E, environment; H, hybrid; A, acacia green manure; C, control; E, early sowing; L, late sowing; H1, C123 × B14A; H2, A632 × W117.

Means for each variable within each fixed factor and without statistical letters are not significantly different, according to Supplementary Table A.8 and the LSD test at $P \leq 0.05$, when followed by different letters statistical differences were found.

aNC means that the LSD cannot be calculated because of the lack of data.

Table 9. Mean comparisons of two hybrids of maize grown in field plots with two fertilization treatments (acacia green manure incorporated into the soil 4 months before sowing *vs* control) for each environment in experiment 3 (2019)

Environment	Fixed effects	Category	Number of plants (sub-plot)	Total plant dw (kg)	Ear dw (kg)	Stalk dw (kg)	Ear dw/total dw ratio	Number of ears (plot)	Grain dw (g)	100k dw (g)
Early sowing	T	Acacia green manure	4.0 ± 0.63	0.04 ± 0.012	0.002 ± 0.002	0.009 ± 0.002	0.019 ± 0.017	0.1 ± 0.1	1.7 ± 1.7	4.5 ± 4.51
		Control	4.1 ± 0.64	0.06 ± 0.020	0.001 ± 0.001	0.014 ± 0.004	0.008 ± 0.007	0.1 ± 0.1	0.8 ± 0.8	3.2 ± 3.2
		LSD	–	–	–	–	–	–	–	–
	H	1118	4.1 ± 0.64	0.05 ± 0.014	0.002 ± 0.002	0.012 ± 0.002	0.019 ± 0.017	0.1 ± 0.1	1.7 ± 1.7	4.5 ± 4.51
		1252	4.0 ± 0.63	0.05 ± 0.020	0.001 ± 0.001	0.011 ± 0.004	0.008 ± 0.007	0.1 ± 0.1	0.8 ± 0.8	3.2 ± 3.2
		LSD	–	–	–	–	–	–	–	–
	T × H	EA H1	3.8 ± 1.25	0.05 ± 0.025	0.004 ± 0.004	0.013 ± 0.005	0.044 ± 0.038	0.3 ± 0.25	3.3 ± 3.30	9.0 ± 9.02
		EC H1	4.5 ± 0.50	0.05 ± 0.017	0.000 ± 0.000	0.011 ± 0.003	0.000 ± 0.000	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
		EA H2	4.3 ± 0.48	0.03 ± 0.003	0.000 ± 0.000	0.007 ± 0.001	0.000 ± 0.000	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
		EC H2	3.8 ± 1.25	0.07 ± 0.040	0.003 ± 0.003	0.017 ± 0.008	0.019 ± 0.016	0.3 ± 0.25	1.6 ± 1.61	6.5 ± 6.45
Late sowing	T	Acacia green manure	5.0 ± 0.00	0.26 ± 0.062	0.140 ± 0.050	0.052 ± 0.012	0.462 ± 0.056 b	3.6 ± 0.38	110.0 ± 40.5	25.8 ± 0.69 a
		Control	5.0 ± 0.00	0.48 ± 0.087	0.304 ± 0.063	0.097 ± 0.018	0.613 ± 0.034 a	4.5 ± 0.33	236.0 ± 55.4	22.5 ± 1.15 b
		LSD	–	–	–		0.146	–	–	2.953
	H	H1	5.0 ± 0.00	0.42 ± 0.082	0.240 ± 0.066	0.084 ± 0.016	0.504 ± 0.057	4.5 ± 0.27	191.0 ± 56.2	24.6 ± 1.03
		H2	5.0 ± 0.00	0.32 ± 0.087	0.204 ± 0.063	0.064 ± 0.017	0.570 ± 0.048	3.6 ± 0.42	155.0 ± 50.9	23.7 ± 1.21
		LSD	–	–	–	–	–	–	–	–
	T × H	LA H1	5.0 ± 0.00	0.28 ± 0.063	0.129 ± 0.050	0.056 ± 0.013	0.416 ± 0.080	4.0 ± 0.41	98.0 ± 44.5	25.4 ± 1.29
		LC H1	5.0 ± 0.00	0.57 ± 0.116	0.351 ± 0.096	0.113 ± 0.023	0.593 ± 0.061	5.0 ± 0.00	285.0 ± 83.5	23.7 ± 1.67
		LA H2	5.0 ± 0.00	0.24 ± 0.117	0.151 ± 0.096	0.049 ± 0.023	0.508 ± 0.082	3.3 ± 0.63	123.0 ± 74.7	26.1 ± 0.68
		LC H2	5.0 ± 0.00	0.40 ± 0.133	0.258 ± 0.087	0.080 ± 0.027	0.633 ± 0.036	4.0 ± 0.58	188.0 ± 76.0	21.2 ± 1.56

Mean values ± standard errors are shown.
dw, dry weight. T, treatment; H, hybrid; A, acacia green manure; C, control; E, early sowing; L, late sowing; H1, C123 × B14A; H2 = A632 × W117.
Means for each variable within each fixed factor and without statistical letters are not significantly different, according to Supplementary Table A.9 and the LSD test at $P \leq 0.05$, when followed by different letters statistical differences were found.

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Table 10. Mean comparisons for soil nutrients of filed plots where maize was grown with two fertilization treatments (acacia green manure incorporated into the soil 4 months before sowing *vs* control) in two environments in experiment 3 (2019)

	Treatment (T)			Environment (E)			E × T					
Response variable	Acacia green manure	C	LSD	E	L	LSD	EA	EC	LSD	LA	LC	LSD
N at T0 (%)	0.23 ± 0.010 a	0.22 ± 0.009 b	NC[a]	0.20 ± 0.003 b	0.26 ± 0.003 a	NC	0.20 ± 0.006	0.20 ± 0.003	–	0.26 ± 0.003	0.25 ± 0.003	–
C at T0 (%)	3.01 ± 0.210 a	2.81 ± 0.203 b	NC	2.29 ± 0.033 b	3.60 ± 0.087 a	NC	2.33 ± 0.058	2.24 ± 0.026	–	3.69 ± 0.079	3.50 ± 0.160	–
NO_2^- at T0 (mg kg^{-1})	0.153 ± 0.026	0.135 ± 0.031	–	0.086 ± 0.016 b	0.208 ± 0.026 a	NC	0.112 ± 0.028	0.060 ± 0.009	–	0.194 ± 0.38	0.224 ± 0.039	–
NO_3^- at T0 (mg kg^{-1})	2.17 ± 0.469 a	1.76 ± 0.218 b	NC	2.99 ± 0.239 a	0.864 ± 0.137 b	NC	3.68 ± 0.202 a A	2.30 ± 0.140 b B	0.55	0.66 ± 0.124 c	1.11 ± 0.228 c	NC
NH_4^+ at T0 (mg kg^{-1})	37.1 ± 6.94	30.4 ± 5.63	–	51.3 ± 4.35 a	14.8 ± 1.47 b	NC	58.8 ± 4.34	43.9 ± 6.48		15.4 ± 2.03	14.2 ± 2.35	
N at T1 (%)	0.23 ± 1.013	0.24 ± 0.012	–	0.20 ± 0.006 b	0.27 ± 0.005 a	0.02	0.20 ± 0.009	0.21 ± 0.008	–	0.27 ± 0.009	0.28 ± 0.006	–
C at T1 (%)	2.89 ± 0.213	2.90 ± 0.229	–	2.22 ± 0.051 b	3.57 ± 0.109 a	0.26	2.23 ± 0.093	2.20 ± 0.053	–	3.55 ± 0.135	3.60 ± 0.183	–
NO_2^- at T1 (mg kg^{-1})	0.207 ± 0.019	0.210 ± 0.033	–	0.172 ± 0.016	0.245 ± 0.030	–	0.182 ± 0.017	0.161 ± 0.029	–	0.231 ± 0.032	0.259 ± 0.053	–
NO_3^- at T1 (mg kg^{-1})	1.87 ± 0.41 b	16.80 ± 4.25 a	7.47	13.80 ± 4.70 a	4.90 ± 1.62 b	7.47	2.28 ± 0.80 b B	25.30 ± 6.62 a A	14.86	1.46 ± 0.13 b X	8.33 ± 2.61 b Y	5.81
NH_4^+ at T1 (mg kg^{-1})	7.5 ± 1.34 b	67.1 ± 24.1 a	40.46	63.4 ± 24.6 a	11.3 ± 3.87 b	40.46	8.0 ± 2.72 b B	119.0 ± 37.90 a A	84.73	7.1 ± 0.65 b	15.5 ± 7.63 b	17.5
N at T2 (%)	0.21 ± 0.012	0.22 ± 0.010	–	0.19 ± 0.005 b	0.25 ± 0.007 a	0.02	0.18 ± 0.008	0.20 ± 0.005	–	0.25 ± 0.011	0.25± 0.012	–
C at T2 (%)	2.77 ± 0.184	2.87 ± 0.190	–	2.26 ± 0.047 b	3.38 ± 0.106 a	0.25	2.22 ± 0.059	2.31 ± 0.073	–	3.32 ± 0.154	3.44 ± 0.156	–
NO_2^- at T2 (mg kg^{-1})	0.010 ± 0.019 b	0.132 ± 0.015 a	0.03	0.071 ± 0.010 b	0.161 ± 0.013 a	0.03	0.050 ± 0.010	0.091 ± 0.013	–	0.150 ± 0.023	0.173 ± 0.012	–
NO_3^- at T2 (mg kg^{-1})	12.4 ± 2.06	25.7 ± 6.04	–	15.8 ± 2.59	22.2 ± 6.33	–	11.6 ± 2.88	20.0 ± 3.74	–	13.2 ± 3.10	31.3 ± 11.6	–
NH_4^+ at T2 (mg kg^{-1})	9.5 ± 0.80	10.3 ± 0.47	–	9.3 ± 0.40	10.4 ± 0.82	–	9.0 ± 0.67	9.6 ± 0.46	–	9.9 ± 1.5	10.9 ± 0.76	–

Mean values ± standard errors are shown.

Means for each variable within each fixed factor and without statistical letters are not significantly different, according to Supplementary Table A.10 and the LSD test at $P \leq 0.05$, when followed by different lowercase letters statistical differences were found. Different capital letters refer to significant differences between treatments within each maize sowing (within interaction factor) according to significant P values from Supplementary Table A.11 and LSD test at $P = 0.05$.

A, acacia green manure; C, control; E, early sowing; L, late sowing. T0, incorporation of acacia green manure 4 months before sowing maize. T1, application of conventional fertilization to control treatment and maize sowing. T2, harvesting maize and end of the experiment.

aNC means that the LSD cannot be calculated because of the lack of data.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1742170521000570

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Data availability statement. The data that support the findings of this study are available from the corresponding author, P.L., upon request.

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