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RESEARCH

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# Combined effects of landscape composition and pesticide use on herbivore and pollinator functions in smallholder farms

Quentin Struelens<sup>1,2\*</sup> , Diego Mina<sup>3</sup> and Olivier Dangles<sup>2,3</sup>

## Abstract

**Background:** Landscape composition has the potential to foster regulating ecosystem services such as pollination and biocontrol in temperate regions. However, most landscape studies do not take pesticide use into account even though it is the main control strategy worldwide and has negative impacts on beneficial insects. Moreover, few studies have explored these combined effects in smallholder cropping system with diverse landscapes and small cultivated fields.

**Methods:** We assessed the effect of semi-natural cover and pesticide use on pollinator and herbivore abundances and functions in 9 fields in the Ecuadorian Andes through participatory experiments with smallholder farmers. We performed a path analysis to quantify the effects of landscape and pesticide use on herbivory, pollination and ultimately yield.

**Results:** Pesticide use significantly reduced pollinator abundance but had no significant effect on pest abundance. Similarly, we found non-significant effects of landscape composition on either herbivory and pollination. The study also provides new information on understudied Andean lupine's pests and pollinators, whose application for small farmers is discussed. Finally, we hypothesize that peculiarities of tropical smallholder cropping systems and landscapes could explain the non-significant landscape effects on insect-based processes, which calls for more research in places outside the well-studied temperate region.

**Conclusions:** Landscape composition did not show any significant effect on pest and pollinator while pesticide use decreased the abundance pollinators, but with no significant effect on yield. This study also provides information about Andean lupine reproduction and overcompensation mechanisms that could be of interest for local farmers and researchers of this understudied crop.

**Keywords:** Andean lupine, Biological control, Ecosystem services, Exclusion experiment, Insecticide use, Participatory research

## Introduction

Smallholder farmers in developing countries are heavily dependent on insecticides for herbivorous pest control (Schreinemachers and Tipraqsa 2012). Most of

these insecticides have a broad spectrum of action (e.g. organophosphates, carbamates) and can therefore harm non-target organisms (Gill and Garg 2014). Even sublethal doses have detrimental effects such as disturbing foraging behaviour and memory abilities, reducing longevity and immunity (Desneux et al. 2007; Blacquièrre et al. 2012; Gill et al. 2012). Among the non-target insects, some are beneficial for crop production, such as natural enemies and pollinators. When natural enemies

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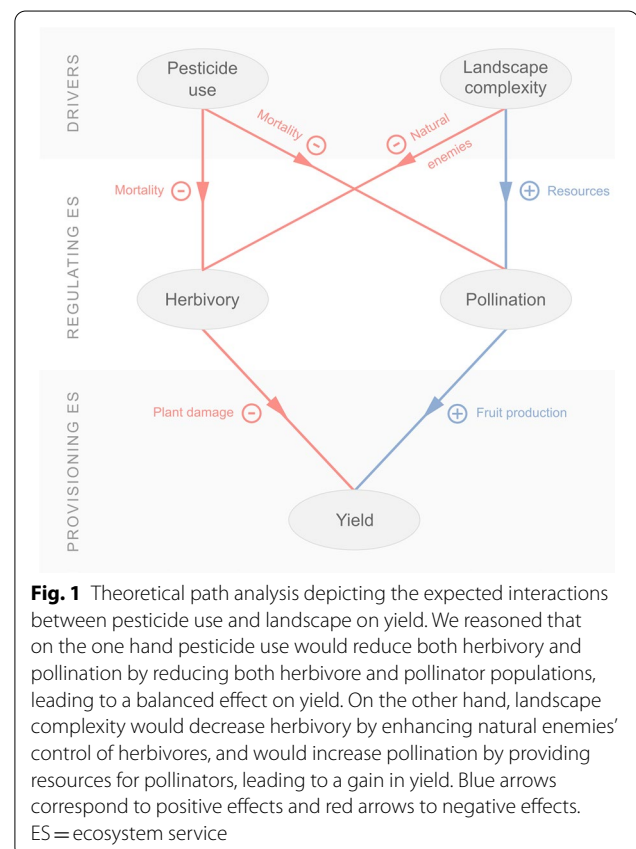
(predators, parasitoids or entomopathogens) are killed by pesticides but a moderate number of pests survive, pest outbreaks may occur due to a decrease in biological pest control (Dutcher 2007). When pollinators are killed by pesticides, it reduces the pollination service for the crop and triggers a reduction in yield, if the crop is dependent on animal-mediated pollination. Therefore, pesticide use against a phytophagous insect can lead to undesired adverse consequences on both biocontrol and pollination.

Increasing the natural habitats surrounding crop fields has been proposed as an alternative solution to herbivore control while mitigating the negative impacts of pesticides (Landis et al. 2000; Bianchi et al. 2006). With a higher diversity of natural and cropped areas in the landscape, natural enemies are more likely to find suitable resources (host plant, nectar...) when compared to monocultures (Gurr et al. 2017). Natural habitats also provide resources for pollinators, thereby enhancing in-field pollination and potentially crop production (Ricketts et al. 2008). Overall, increasing natural habitat in landscape surrounding the fields has been correlated in many cases with higher biocontrol and pollination services (Shackelford et al. 2013), even though few data exist from tropical regions (Poveda et al. 2012; Haan et al. 2020).

However, the majority of research studying the effect of surrounding landscape on agricultural ecosystem services (ES), such as pollination or biocontrol, have been carried out in fields where no pesticide was sprayed (see review in Veres et al. 2013), which may not be realistic. Indeed, both mechanisms (pesticide use and landscape provision of ecosystem services) occur and interact together, as it is the case in smallholder cropping systems. Therefore most of these agricultural landscapes encompass a mosaic of sprayed and unsprayed areas, combined with (semi-)natural and cropped areas. Recent studies have thus focused on understanding how the interaction between landscape and pesticide use impacts biological control (Ricci et al. 2009, 2019; Gagic et al. 2017, 2019). Nevertheless, few studies have included pollination in the analysis, even though it is also impacted by both landscape and pesticide use (but see Samnegård et al. 2016) and to our knowledge none in a tropical setting.

Thus, there is a need to understand how landscape composition and pesticide interact and affect pollination, herbivory and ultimately yield. We chose to focus on the two lower levels of the biocontrol tritrophic interactions (natural enemy-herbivore-yield) because the effect of landscape on natural enemies does not necessarily translate into an effect on herbivory or yield (Chaplin-Kramer et al. 2011). Moreover, yield is rarely considered in landscape-based biocontrol studies (Veres et al. 2013; in 14% of the responses included in

the review by Karp et al. 2018), yet, this is an important metric for farmers. We therefore established a conceptual framework detailing the potential effect of landscape structure and pesticide use on yield, through their combined effects on herbivores and pollinators (Fig. 1). This framework relies on two main hypotheses. On the one hand, we reasoned that a more diverse surrounding landscape would result in a higher yield, through an increase in pollination and a decrease in herbivory. On the other hand, we predicted a more balanced effect of pesticide use on yield, through a decrease in both pollination and herbivory (Fig. 1). In this study, we tested these hypotheses using lupine grown in the tropical Andes as a study model. Our specific objectives were (i) to assess the combined effect of landscape and pesticide on both pollination and herbivory, (ii) to measure both the abundances and functions of pollinators and herbivores, and (iii) to quantify the effect on yield. Smallholder agricultural landscapes are relevant and realistic models because they incorporate small fields with surrounding semi-natural habitats and a diversity of pesticide management strategies.



## Materials and methods

### Study crop

This study focuses on the Andean lupine (*Lupinus mutabilis* Sweet), a legume crop cultivated in the tropical Andes for its edible beans rich in proteins. This crop was once extensively cultivated by pre-Incan civilizations, then became supplanted mostly by fava beans during the Spanish colonization (Cowling et al. 1998). However, in the early 2000's the Ecuadorian government promoted its cultivation, leading to a renewed interest by farmers and consumers (Nicklin et al. 2006). In this context, Andean lupine crops are now found over a gradient of agricultural intensification, reflected in the surrounding landscape complexity and crop management. The expansion of Andean lupine cultivation in Ecuador went along with an increase in predation by herbivore invertebrates (Caicedo and Peralta 2000). Among these herbivores, the apex fly borer (Anthomyiidae, see below for taxonomic details) is ubiquitous in lupine crops and attacks the plant throughout its whole lifecycle (Mina et al. 2017). Pesticide application is the main option available for Ecuadorian farmers to control pest populations. Therefore, Andean lupine is a suitable crop to study the combined effect of pesticide use and landscape complexity in tropical latitudes, as it grows along a gradient of landscape complexity and pesticide use.

### Study sites

The study was carried on within 30 fields owned by local farmers between January and October 2017. Field selection was a compromise between the need to include different communities with different practices and landscape compositions, and farmers' willingness to participate to the study. We only selected fields sown with "Andino 450" variety of Andean lupine. To obtain the estimates of ecosystem (dis)services (pollination and herbivory) and crop management (pesticide use), we combined experimental and observational approaches with farmer surveys. The number of pesticide applications during the crop lifecycle was determined through interviews with the farmers. A diversity of pesticides was mentioned by the farmers: Acephate, Carbosulfan, Carptap, Deltamethrin and Chlorfenapyr.

### Landscape-scale analysis

Orthophotos of the landscape surrounding each field were obtained by flying a drone (DJI Phantom Pro 4, 20Mpx camera 4:3 ratio, 4864 × 3648px resolution, RGB channels; Fig. 2a). We used Pix4Dcapture software at 200 m altitude with 60% overlap between shots, covering a circle of 400 m radius around the field (50 ha). In landscapes with large differences in altitude we flew the drone at 300 m to avoid any image distortion. An ortho-mosaic

image was generated by Pix4D software that combined 81 shots for each field (see Faye et al. 2016). We down-scaled the images to a 40 cm/pixel resolution. Fields were selected at least 1 km away from each other to avoid any overlap between surrounding landscapes.

To determine land use cover we defined a classification scheme (Additional file 1: Table S1) with 15 classes based on the knowledge of the study areas and local crop rotations. Land use was determined by visual inspection of the orthophotos. We used Random Forest algorithm (ntree = 500) to classify the pixels based on RGB channels (Hayes et al. 2014). We manually drew a set of polygons for each land use on every image (ranging from 67 to 122 polygons per image; Additional file 1: Table S2). These polygons were randomly separated into a calibration dataset (70%) used to calibrate the classification model and an evaluation dataset (30%) to evaluate the model classification ability (Additional file 1: Table S3). We decided to use the fallow and grassland classes as a proxy of the semi-natural cover because this is where most of the non-crop flowering plants are naturally growing. Indeed, in the Andean inter-valleys, the tree rows surrounding the crops are mainly composed of eucalyptus and pines where few flowers grow (pers. obs). The area of semi-natural cover in the surrounding landscape of the 11 study fields ranged from 20% of up to 67% (Additional file 1: Table S4).

### Monitoring of insect populations

Within each lupine field, the abundance of pollinators and herbivores was estimated using three pitfall and three colour traps. We chose a participatory monitoring approach, meaning that the setup and gathering of the traps were carried out by the farmers themselves (and their relatives) after being thoroughly trained (Fig. 2b). The colour trap consisted of a yellow bowl (40 cm in diameter and 20 cm deep) and the pitfall trap of a plastic cup (8 cm in diameter and 15 cm deep) filled with water and a few drops of soap. All the trapped insects were preserved in 70% ethanol from collection to identification in the lab. The traps were set up in the fields from March to July 2017, and the time between set-up and collection varied between 6 and 64 days (mean = 24 days) due to variations in farmers' availability. To account for this uneven sampling, we divided the observed abundances by the number of days corresponding to the trapping time. Then, the mean abundance of pollinators and apex borers were averaged across traps. Lupine apex borer fly abundance was considered as a good estimate of herbivore abundance because (i) it is a widespread herbivorous insect of concern for farmers, (ii) it feeds throughout the whole crop cycle, and (iii) its damage is easily visible through plant architecture changes. In order



**Fig. 2** Pictures showing the diversity of methods used in this study. **a** Drone acquisition of landscape images. **b** Participatory monitoring of insects with local farmers. **c** Insect exclusion experiments **d** Farmer spraying pesticides and Andean landscape in the background

to determine the taxonomic status of the lupine apex borer fly, we extracted DNA from one specimen and performed a DNA barcoding on COI gene following widely used protocols (Morgulis et al. 2008). We identified the morphospecies as being a member of the Anthomyiidae family, potentially from the genus *Lasiomma*. We characterized pollinators by taking into account all hymenoptera belonging to the Apidae family as it is the only group for which the pollination role was ascertained (Roubik 1995; Ochoa-Zavala et al. 2016). We could not include natural enemies within our framework because of the lack of knowledge about the lupine apex borer's natural enemies. Overall, entomofauna in the Andean inter-valley is largely unknown and has not been described (e.g. Mina et al. 2017), which makes insect identification difficult. Compared to extensive and long-term research in the Amazonian region (e.g. since Bates 1862), it is

surprising to witness that most of the less-diverse entomofauna in the Andes remains largely unknown.

#### Exclusion experiments

We used in situ exclusion experiments to estimate herbivory and pollination in the focal crop within the landscape (Fig. 2c). Exclusion experiments allow to compare the sole effect of herbivory or pollination in fields scattered across a large geographical range, neutralizing the differences in abiotic conditions between fields (climate, topography, soil texture, etc.; Chaplin-Kramer et al. 2011). To estimate herbivory, three cages were installed around randomly selected lupine plants in each field to exclude all herbivores from germination to flowering. Several seeds per hole are traditionally sown, and the density of plants varied across cages depending on the farmer's practice. Cages consisted in a wooden frame of

1 m high, a top cross of  $0.5 \times 0.5$  m, wrapped with a 0.36 mm mesh-size tulle covering  $1 \times 1$  m surface at the ground level. To estimate herbivore damage we measured for every plant within the cage the Apical Dominance Index (ADI) = number of ramifications / stem height. This index reflects the dominance of the apical meristem compared to lateral meristems and the resulting plant architecture (Pérez-Harguindeguy et al. 2013). The ADI of each excluded plant was compared with the ADI of one non-excluded plant randomly selected within a 2 m radius from the excluded plant to compute the  $\Delta\text{ADI} = \text{ADI}_{\text{excluded}} - \text{ADI}_{\text{non-excluded}}$ , reflecting the estimated herbivory for each field. All plants were selected at least 5 m away from the field limits to avoid any border effect. The exclusion of phytophagous insects from plant germination until flowering (four to five months) represented a major challenge because the cages were vulnerable to several adverse conditions (strong winds, destruction by wildlife...). Nevertheless, this exclusion time is long compared with most studies in the literature that generally focus on short generation-time insects (e.g. aphids; see list in Karp et al. 2018). Even though the sample size may seem low, it is similar with other studies on this topic (e.g. Chaplin-Kramer et al. 2011; Thies et al. 2011). Temperatures outside and inside the cage were recorded during one week which resulted in a mean temperature inside the cage 1.12 °C higher than outside. This difference in temperature is lower than the natural thermal variability measured within one field (Faye et al. 2016).

Within the same fields, we randomly selected two plants not included in the herbivore exclusion experiment, for which two inflorescences were excluded from pollinators. The cage was made with a tulle with mesh size of 0.36 mm, with a height of 50 cm and a width of 30 cm. The pollinators were excluded from pre-flowering to harvest. To estimate pollination we counted the number of developed and aborted ovules per pod and computed a Developed Seed Index (DSI) = number of developed ovules / (number of developed ovules + number of aborted ovules). The DSI of non-pollinated inflorescence were compared with the opposite inflorescence from the same plant where pollinators were allowed, in order to compute the  $\Delta\text{DSI} = \text{DSI}_{\text{pollinated}} - \text{DSI}_{\text{unpollinated}}$ .

Field yield was estimated by harvesting all the seeds from three randomly selected plant groups covering  $1 \text{ m}^2$  surface (plants emerging from the same seed hole). The seeds were dried for 48 h at 80 °C and their dry biomass was weighted (precision of 0.1 g) (Pérez-Harguindeguy et al. 2013). We computed the yield (kg/ha) = plant group dry biomass (kg)  $\times 1 \text{ (m}^2) \times 10\,000 \text{ (m}^2)$ .

### Statistical analyses

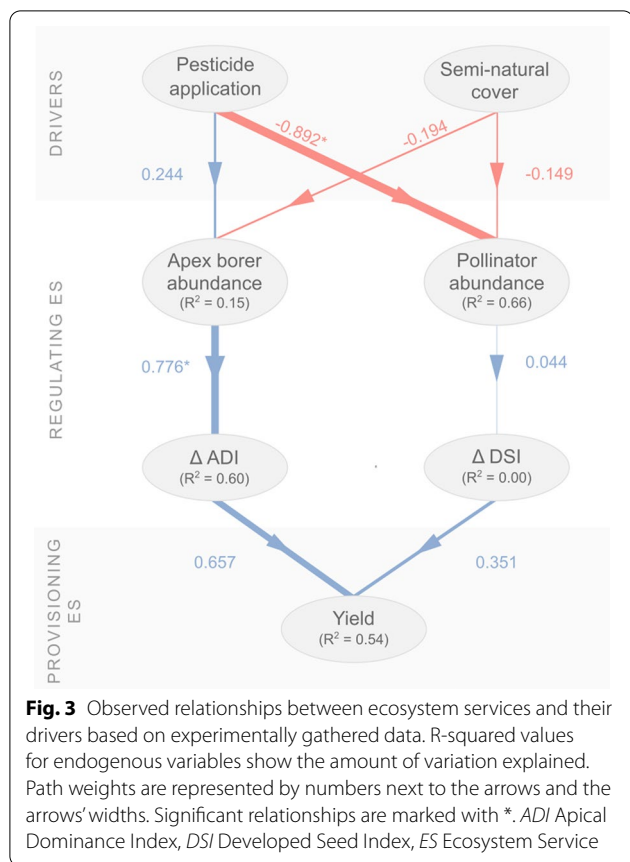
From the 35 initial fields selected, 9 were left with a complete set of data at the end of the experiment, reflecting the difficulties associated with participatory research. Indeed, 18 farmers lost interest in the participatory monitoring of insects. Exclusion experiments were carried out within the 17 fields left but in 6 fields the cages were damaged or disappeared. We therefore flew the drone in the 11 fields where the cages were not damaged and where insects were still monitored by the farmers. Finally, pesticide use information was missing for 2 fields where farmers were unreachable.

We constructed a path analysis to explore the relationships of landscape and pesticide use on herbivory, pollination and ultimately yield (Fig. 1). Path analysis is a particular case of structural equation model with inner model only, that estimates direct, indirect, and total effects of explanatory variables on dependent variables to describe correlations among them (Sanchez 2013). Path analysis is used to explore theoretically designed hypothesis. In path analysis variables are either exogenous (e.g. drivers) or endogenous (e.g. ecosystem service indicators) depending on hypothesized pathways. The exogenous variables included were the proportion of semi-natural cover and the number of pesticide applications while the endogenous variables were the field yield, the abundance of apex borer, the abundance of pollinators, the ADI and the DSI. R-squared values for endogenous variables show the amount of variation explained. The path coefficients depict the correlation between two variables. We assessed the differences in mean ADI and DSI between excluded and non-excluded plants using two Generalized Linear Mixed Models (GLMM) with field as random effect. For ADI, we used a Gamma error distribution to account for the long tail distribution. For DSI we used a Binomial error distribution with a logit link function to account for the proportional nature of this index, and we also used the total number of seeds to weight the GLMM.

Statistical analyses were performed using R v3.5.1 (R Core Team 2018), the path analysis was performed using the *plspm* R package (Sanchez 2013) and the GLMM using *glmer* function from the *lme4* package.

### Results

Our results revealed only two significant relationships from our hypothesized path analysis (Fig. 3). First, we found significant negative effect ( $p=0.021$ ; path-coefficient = -0.892) of the number of pesticide applications on pollinator abundance (Fig. 3), suggesting a high pollinator mortality due to tested pesticide use. Second, a positive significant effect ( $p=0.014$ ; path-coefficient = 0.776; Fig. 3) arose between apex borer



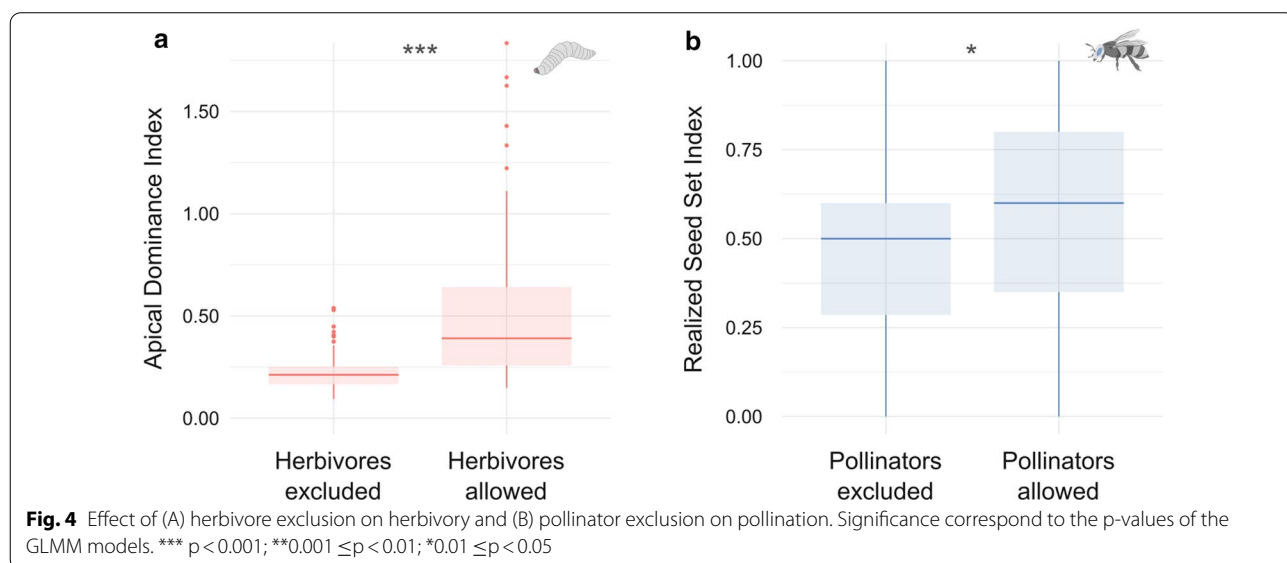
abundance and ADI, reflecting the capacity of apex borer to modify plant architecture. This hypothesis was also supported by the significantly lower herbivory for plants excluded from insects ( $p < 0.001$ ; Fig. 4a,

Additional file 1: Fig. S3, Table S4) with a mean ADI 25.7% higher compared to excluded plants. We also found a significant effect of pollinator exclusion on pollination ( $p = 0.017$ , Fig. 4b, Additional file 1: Fig. S4, Table S5), with non-excluded flowers exhibiting a mean DSI higher by 10.5% compared to excluded flowers. All the other relationships depicted in the path analysis showed non-significant effects (Fig. 3).

### Discussion

Natural habitat conservation at the landscape scale has been proposed as another pest management strategy that can be integrated with pesticide use, with synergistic benefits for pollination (Ricketts et al. 2008; Gurr et al. 2017). Therefore, sprayed crops and semi-natural areas are embedded within the same landscape, interacting together. Our study documents a case of the combined effect of these two drivers in a smallholder farming system in the Ecuadorian Andes, with small size fields (< 1-2 ha) and diversified crops.

From our initial sample of 30 farms, we were able to obtain complete data for only 9 farms due to issues during our participatory monitoring of insects, and to the long time-scale of the exclusion experiments (during the whole crop cycle, see also Dangles et al. 2010 for a discussion on benefits and drawbacks of crop pest participatory monitoring). Therefore, most of our non-significant results could be due to the low sample size we ended with. We nevertheless discuss our significant results and explore alternative hypothesis for non-significant effects with potential biological mechanisms at work.



### Pesticide effect on pollinators and pests

Our study showed a significant negative effect of pesticide applications on pollinator abundance (Fig. 3). While similar results have been reported in intensified cropping systems (e.g. Johnson et al. 2010; de Oliveira et al. 2019), there are fewer support of this process in smallholder farms where application occurs at a reduced scale, with weaker farmer's knowledge of the products and their hazards (e.g. Mengistie et al. 2017). Pollinators play a key role because 35% of the global food production is dependent on animal pollination (Klein et al. 2007). However major crop production in the Andes may be more resistant to pollinator disappearance than other regions because some of the major crops grown here are not dependent on animal pollination (e.g. maize, potato, quinoa; Appendix 1 and 2 in Klein et al. 2007). While pesticide use significantly decreased pollinator abundance, no significant effect of pesticide could be found on pest abundance. We hypothesize that this result could be linked with the ecology of the pest as only a small amount of pesticide can reach it within its protective refuge inside the stem.

### Effects of landscape composition on pollinators and pests

While pesticide had a significant effect on pollinators, our results showed only non-significant effects of the proportion of semi-natural cover surrounding the field on abundance of both pests and pollinators (Fig. 3). The underlying biological mechanism behind our initial hypothesis was that more floral resources (e.g., shelter and nectar) were present in semi-natural habitat than cultivated crops, which would be beneficial to both natural enemies (Gurr et al. 2017) and pollinators (Ricketts et al. 2008). However, this hypothesis was not supported by our results. This finding could be related with the small field size and the crop heterogeneity within the landscape compared to temperate areas. Indeed, Sirami et al. (2019) showed that reducing field size and increasing crop diversity lead to similar benefits for biodiversity and related ecosystem services than increasing semi-natural habitat cover. Moreover, the proportion of semi-natural habitat in our landscapes were high (20–60%) compared to ranges commonly found in the literature (Sirami et al. 2019). Thus, we suggest that these farming systems may not benefit from an additional increase in semi-natural habitat because the current traditional farming practices already foster a relatively high heterogeneity of crop and conservation of semi natural habitats within the landscape. Furthermore, in the tropical region, crop spatial heterogeneity is even higher because of an additional phenological heterogeneity (i.e. crops at different lifecycle stages) allowed by the absence of seasonality.

We believe that these aspects should be further explored in smallholder farm systems to verify this hypothesis.

### Effects of pollination on Andean lupine

Our results also showed that flower visitation by insects increased the mean seed set by 10.5% (Fig. 4b). This is consistent with what has been found for other species of lupine, which display a combination of allogamous (through cross-pollination) and autogamous reproductions (Cowling et al. 1998). However, the cross-pollination rate has been shown to be highly variable among species and individuals, depending on genetic and environmental factors such as plant variety and distance to conspecifics (Cowling et al. 1998). For *Lupinus mutabilis*, Cowling et al. (1998) reported a cross-pollination rate of 4 to 11%, while experiments conducted by Caligari et al. (2000) found an outcrossing rate reaching up to 58.8%. Our results add up to the literature, suggesting that allogamous reproduction in *L. mutabilis* has a significant impact on increasing fruit production. We also provide the first evidence that seed production of the variety “450-Andino” of Andean lupine, the dominant variety used as a crop in Ecuador, is increased by pollination. Although pollination increased seed production, the abundance of pollinators had almost no effect on pollination (Fig. 3). This surprising result could be due to the fact that Apidae family may not be the main pollinator of Andean's lupine in our study area compared to study carried out in Peru (Ochoa-Zavala et al. 2016). Another explanation could be linked with the “flower tripping” pollination mechanism occurring in other lupine species (Williams 1987). Flower tripping is a self-pollination triggered by the movements (landing, take-off) of large insect within the flower, resulting in a very generalist pollination mechanism. If this mechanism takes place in Andean lupine, it could explain why the abundance of Apidae only had no effect on fruit production, because any large generalist insect could pollinate the flowers inadvertently. It is yet unknown whether this mechanism takes place in Andean lupine reproduction, and additional studies should focus on this aspect to shed light on this understudied part of its ecology, for example by manually simulating flower tripping events.

### Effect of pest on Andean lupine architecture and yield

A positive significant effect was observed between apex borer abundance and the apical dominance index, indicating that this pest is able to transform Andean lupine's architecture (Fig. 3). Indeed, the fly's larvae feed on the internal tissues of the plant and frequently damages apical meristems. In response, the Andean lupine plant

stimulates its lateral meristems growth, resulting in a significant change in plant architecture to compensate the loss induced by the pest (Fig. 4a). When the compensation exceeds the loss, a mechanism called overcompensation takes place, leading to a higher growth than if the plant was left undamaged. Overcompensation can be either reproductive (increased fruit production) or vegetative (increased vegetative growth). Even though reproductive overcompensation seems widespread for species in the Fabaceae family (Garcia and Eubanks 2018), our results do not support this hypothesis as herbivory did not significantly increase yield. Nevertheless, a case of reproductive overcompensation for *L. mutabilis* was previously reported in the literature (Porter 1982), where overcompensation was induced in an opposite manner (cutting lateral branches increased pod set in apical flowers). Overall, our study shows that the apex borer does not significantly increase nor decrease Andean lupine's yield, which suggests that the plant is able to compensate for the damage taken. Therefore, even though the apex borer's damage is very conspicuous due to the plant's architecture modifications, our results suggest that this species may not be as detrimental to crop production as farmers currently perceive.

### Limitations of the study

Several factors that we did not account for, such as tillage, crop rotation, use of fertilizers or timing and modes of action of pesticide could influence the biology of pests and the vulnerability of lupine to pests, and should therefore be further studied. Additionally, our analysis would have benefited from a multiyear experiment because climatic conditions also influence the biology of pests and pollinators. Finally, we believe that more knowledge needs to be gathered about the biology of pests and pollinators associated with Andean lupine because knowing the functional relationships between habitats and insects would help to define functionally meaningful semi-natural habitat to be measured within the landscape.

### Conclusions

This study shows that pesticide use had an overall greater impact on the entomofauna associated with Andean lupine fields than the presence of semi natural habitats nearby. We propose that further studies should explore the role of crop spatial and temporal diversities within the landscape to explain this absence of effect, as the Ecuadorian Andes are very diverse landscapes, both spatially and temporally. Our results also provide information about Andean lupine reproduction and overcompensation mechanisms that could be of interest for local farmers and researchers of this understudied crop.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43170-021-00027-w>.

**Additional file 1.** Additional tables and figures.

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### Authors' contributions

QS and OD designed research; QS and DM performed research and collected data; QS and OD analysed data; QS led the writing with contributions from DM and OD. All authors read and approved the final manuscript.

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### Availability of data and materials

All data necessary to compute the figures are available as supplementary material.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

Authors declare no conflict of interests.

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