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Agroecological consequences of silicon supplementation for a legume cultivation: Two-year-long field observations

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ABSTRACT

Supplementing plants with silicon (Si) often improves plant productivity and resilience to biotic and abiotic stresses, but this is mostly reported in highly controlled experimental environments. The ecological consequences of Si supplementation, including environmental benefits and potential risks, are therefore poorly understood and require field-scale evaluation of how Si supplementation affects the wider ecosystem, such as invertebrate communities and soil physicochemical properties. We conducted the first field assessment of how a legume (lucerne; *Medicago sativa*) agroecosystem and its associated invertebrate communities responded to two levels of Si supplementation (calcium silicate slag), over two years. We quantified seasonal changes in the abundance and diversity of aboveground arthropod communities, crop yield, elemental and nutritional chemistry, and soil pH as well as soil chemistry. The highest rate of Si supplementation increased bioavailable Si in the soil by 181% and soil pH from 5.2 to 6.3, relative to untreated plots, with a significant positive correlation between increased soil bioavailable Si and pH. Si supplementation led to an increase in crop yield by up to 52%; however, the magnitude varied with season. Foliar concentrations of Si tended to increase with Si supplementation, but this increase was marginally significant, potentially due to a dilution effect of higher shoot biomass. Si supplementation did not affect concentrations of most soil elements we quantified or forage quality of lucerne. We recorded over 13,600 arthropods; Si supplementation led to a shift in community structure and overall increased diversity of arthropod functional groups. Notably, the saprophytic fly family, Lauxaniidae was more abundant on Si-supplemented plots compared to untreated plots, potentially due to increased plant turnover. These results indicate that silicon supplementation of a legume agroecosystem, using a by-product of steel production, provides productivity benefits that outweigh some possible detrimental impacts on the ecosystem (i.e. decreased arthropod abundances, toxic metal contamination or reduced forage quality), which we did not detect in our current field study. This management intervention enhances crop yield, so could reduce the need for conventional fertilisers as well as changing soil pH to be more beneficial to crops and some arthropod groups.

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1. Introduction

Achieving food security is key to feed a growing population; approximately three billion people do not have access to healthy diets, contributing to hunger and malnutrition around the globe (FAO et al., 2021), while climate change is already severely impacting the productivity of agricultural ecosystems (Walker and Schulze, 2008; Sharma et al., 2022). Hence, sustainable solutions are urgently required to maintain crop productivity in a changing world (Foyer et al., 2016).

Many legumes (Fabaceae) are highly nutritious because they form a symbiosis with nitrogen-fixing rhizobia inside the root nodules, which convert atmospheric nitrogen into ammonia that can be used by the plant (Peoples et al., 1995). Some leguminous plants, such as beans and peas (known as pulses) are consumed by humans, whereas pasture legumes are used as animal feed (Stagnari et al., 2017). The United Nations (UN) has declared the International Year of Pulses in 2016 (IYP2016), highlighting the essentiality of legumes as sustainable, climate resilient and low-carbon footprint crops, thereby making legumes an ideal candidate to mitigate food and climate crises (www.fao.org). Neglecting the productivity of legume crops, thus can compromise human health, nutritional security and sustainable food production globally (Foyer et al., 2016).

One of the avenues to increase crop productivity is to increase bioavailable silicon in the soil (Tubana et al., 2016). Silicon (Si) is recognised as a plant beneficial nutrient and is also the most abundant metalloid element accounting for 28% by mass in the soil (Epstein, 1999). Nonetheless, only a small fraction of Si in the form of orthosilicic acid (H_4SiO_4) is available for plant uptake, hence supplementation of Si in the soil to increase bioavailable Si for some monocot and dicot crops, including legumes, has been advocated (Owino-Gerroh et al., 2005; Liang et al., 2015; Xu et al., 2020). Si supplementation is needed when bioavailable Si in soils becomes depleted due to intense agricultural turnover (Savant et al., 1997) or naturally low Si-derived soils (e.g. granite and red sandstone) (Liang et al., 2015). Si uptake and accumulation (silicification) can protect plants from various stresses, such as drought, metal toxicity, pathogen and herbivore attacks and consequently, often improves plant productivity (Debona et al., 2017). Silicification involves Si deposition into cell walls and elsewhere, such as within and between cells (Epstein, 1999), and may confer physical resistance against both invertebrate and vertebrate herbivores (McNaughton et al., 1985; Massey and Hartley, 2009). Silicification may also alter plant growth and physiology (Detmann et al., 2012; Vandegeer et al., 2021), potentially impacting organisms that interact with the plant and the wider ecosystem (Cooke and Leishman, 2011b). While most research on the functional role of Si on plants has focused on the grasses (Poaceae), it is increasingly recognised that Si plays a role in other plant taxa, notably legumes (Johnson et al., 2017; Putra et al., 2020, 2021, 2022; Ryalls et al., 2023). For example, Si enrichment resulted in increased plant biomass, nodule abundance, nitrogen-fixing enzyme (nitrogenase) activity and nitrogen fixation in symbiotic legumes. Therefore, Si supplementation may be a feasible method for increasing legume productivity.

It is important to identify economically viable Si sources that increase Si availability in agroecosystems, which could include Si-containing slags (Gascho, 2001). Slag predominantly comprises calcium silicate (Ca_2SiO_4) and is a by-product of iron and steel-mill industries (Wang et al., 2015). Typically, slags contain 14–18% of Si and trace amounts of metals, such as Cu, Zn, Cr, As, Cd, Pb and Hg; the elemental variation depends largely on the original composition of slag materials, and how they are processed (Haynes et al., 2013). Slag supplementation is an effective measure for increasing soil pH (Haynes and Zhou, 2018) and crop yield in sugarcane, rice, tomato, onion, cotton and chilli agroecosystems (Crooks and Prentice, 2017), but little is known about its effects on legumes, especially when soil pH is too acidic as this can impair root nodulation (Ferguson et al., 2013). Next to these potential benefits, risks associated with slag supplementation on agroecosystems should also be anticipated. For example, potentially toxic trace elements associated with slag materials may leach into the soil (Haynes et al., 2013; Haynes and Zhou, 2018; Duboc et al., 2019). Silicification in some grasses could reduce forage digestibility for grazing herbivores (Van Soest and Jones, 1968; Cougnon et al., 2020; Massey and Hartley, 2009).

To date, most studies on Si supplementation take a reductionist approach to studying how plant silicification affects other organisms (e.g. pairwise plant-herbivore or plant-pathogen interactions) and do not explore its impacts on the wider ecosystem, such as arthropod communities and their potential functions. While we lack empirical information from the field, it could be expected that Si supplementation could affect arthropod communities via several mechanisms. These include providing refugia and distinct niches via increased plant biomass, resulting in increased arthropod abundance and diversity (Ryalls et al., 2013); and altering trophic interactions, such as reduced performance of natural enemies due to a poorer quality of the herbivores feeding on Si-supplemented plants (Hall et al., 2021) or increased attraction of natural enemies via changes in herbivore-induced plant volatiles (De Oliveira et al., 2020; Islam et al., 2021; Kvedaras et al., 2010; Liu et al., 2017).

We established a two-year field experiment investigating the impacts of a Si-based fertiliser (blast furnace slag, 'Si' hereafter) supplementation on a lucerne/alfalfa (*Medicago sativa*) agroecosystem. We quantified multiple soil properties: pH and elemental chemistry (C, N, bioavailable N and Si, and other trace elements) and plant properties: yield, foliar elemental chemistry (C, N, Si and Ca) and forage nutritional indices; and the arthropod community (abundance and diversity). We hypothesised that Si supplementation:

(i) increases soil pH and bioavailability of soil Si,
(ii) decreases forage nutritional quality, but increases shoot biomass (i.e. crop yield) due to increased foliar silicification,
(iii) indirectly alters community responses of arthropods, potentially because of Si-induced changes in plant biomass and chemistry, and finally,
(iv) increases concentrations of toxic trace elements, such as Pb and Cd, in the soil due to Si-slag leaching.

2. Materials and methods

2.1. Site description

The experimental site (Fig. 1) was established on an alluvial floodplain at the Hawkesbury Forest Experiment (HFE) on the Hawkesbury campus of Western Sydney University at 25 m elevation (33°36′40′ S, 150°44′26.5′ E). The soils at the HFE (see Barton et al., 2010 for details) are classified as the Clarendon Formation type (Isbell, 2016), an alluvial formation of low-fertility sandy loam soils with low organic matter content, moderate-to-low fertility and low water holding capacity. The soil at the site was low in bioavailable Si (mean ± SE) 23.42 ± 0.83 mg kg^{-1} (see Table S1 in Johnson et al., 2019).

The climate is classified as sub-humid temperate (Barton et al., 2010) with an average annual temperature of 17 °C, a mean maximum temperature of 29 °C in December (warmest month) and a mean minimum temperature of 3 °C in June (coldest month). Frost events occur an average of 13 times per year (Australian Bureau of Meteorology; http://www.bom.gov.au). The long-term mean annual rainfall is 801 mm, with 1st and 9th deciles for rainfall of 528 and 1075 mm, respectively (Barton et al., 2010). According to FAO-56, the ratio of annual precipitation to potential evapotranspiration is approximately 0.6.

2.2. Establishment of experimental plots

In October and December 2017, the experimental site (12 m x 30 m)

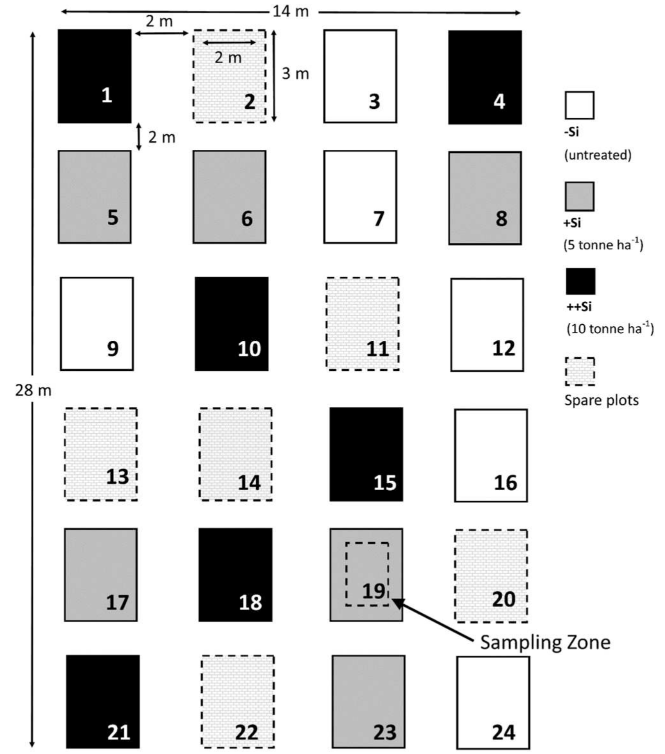


Fig. 1. Configuration and size of experimental plots: white plots indicate untreated or -Si (0 tonne ha^{-1} or 0 kg m^{-2}), grey plots indicate +Si (5 tonne ha^{-1} or 0.5 kg m^{-2}) and black plots indicate ++Si (10 tonne ha^{-1} or 1 kg m^{-2}). Sampling zone (1 ×2 m) is located at the centre of the plots. The other six spare plots (bricks with discontinued lines) were not used for this study.

had vegetation removed. In February 2018, glyphosate (Apparent Glyphosate 450 and Roundup™, Australia) was applied at a rate of 7.2 L ha^{-1}. Additionally, bromoxynil herbicide (Bromicide® 200, Australia) was sprayed at a rate of 1.4 L ha^{-1} three times before Si supplementation started in April 2018. Afterwards, weeding was regularly conducted by hand. Non-Si fertilisers were reapplied twice with 41% K and 17% S (Impact SOP, Elders, Australia) at a rate of 100 kg ha^{-1} and with 20.1% P (Triple Super, Elders, Australia) at a rate of 50 kg ha^{-1} in April 2019 and in March 2020, respectively. The field experiment was conducted between April 2018 (Austral Autumn) and May 2020 (Austral Autumn).

The experimental design included eighteen plots (2 m x 3 m), which were randomly assigned after establishment for Si treatments. Six plots were not treated (controls: **-Si**), six received Si fertiliser at 5 tonne ha^{-1} or 0.5 kg m^{-2} (+**Si**) and six received Si fertiliser at 10 tonne ha^{-1} or 1 kg m^{-2} (++**Si**). Si fertiliser was supplemented in the form of a blast-furnace slag, which largely contains calcium silicate (Ca_2SiO_4) (Plant-Tuff®, Australian Steel Mill Services, Port Kembla, NSW, Australia) by minimal tilling into the soil ca. 5–7 cm deep. This Si fertiliser had a pH of 10.8 and contained Si (17.3%) with akermanite ($Ca_2Mg[Si_2O_7]$) and gehlenite ($Ca_2Al\ [AlSiO_7]$) as dominant minerals (similar Si-based fertiliser product as in Haynes and Zhou, 2018). Configuration and size of experimental plots are provided in Fig. 1.

Irrigation was applied weekly (unless it rained) or adjusted depending on the frequency of rainfall using the single "Knock" sprinkler and "pop-up" sprinklers on an automatic controller. Di-ammonium phosphate (DAP: 18% N, 20.2% P and 1.5% S) fertiliser was applied once at a rate of 100 kg ha^{-1} prior to seeds sowing in April 2018. Experimental plots were sown with lucerne, *Medicago sativa* (cv. Sequel) seeds at a rate of 15 kg ha^{-1}. Seeds were previously inoculated with a rhizobial strain *Ensifer meliloti* RRI128 ("Nodule N" NewEdge Microbials, Albury, NSW, Australia) containing 250 g inoculant dissolved in 800 mL water, following the product instruction on the package. The cultivar Sequel was chosen because it is active during winter months and is moderately resistant to pests and diseases (Bray and Irwin, 1998).

2.3. *Field sampling campaign*

Lucerne was harvested periodically during the early flowering period; soil collections and measurements were taken at six monthly intervals. Repeated shoot harvests for lucerne were done because it is important to realise the dynamic effects of Si supplementation on yield and other plant traits not only at a single time point, but also across multiple time points particularly on a perennial forage species. To avoid excessive disturbances on the experimental plots, quality checks for nodulation were randomly conducted once at the first harvest of Si-untreated plants from the six spare plots. Effective nodulation in the field was confirmed by the presence of characteristic pink nodules of the commercial rhizobial inoculant *E. meliloti* RRI128, that was also previously used in Johnson et al. (2017) and Johnson et al. (2018a). The full details of field sampling campaign are provided in Table 1. To avoid edge effects, vegetation, arthropod and soil sampling were conducted on the central zone (1 × 2 m) of each plot as shown in Fig. 1.

2.4. *Vegetation collection and yield*

Lucerne at 5 cm aboveground was cut using razor clippers in 1 m^2 quadrats on the central sampling zone, freshly weighed on a two-digit balance, stored in paper bags (200 × 270 mm) and oven-dried at 70 °C for 72 hours. Oven-dried shoots were then weighed on a two-digit balance to obtain the dry biomass of vegetation as a proxy for crop yield.

2.5. *Soil samples*

Twelve soil cores were taken from each plot using a soil corer (1.5 cm diameter and 30 cm length) to obtain enough material for a wider suite of chemical analyses (Table 1). Three cores per grid of each plot were taken from the periphery of the sampling zone (Fig. 1) to avoid disturbance of the vegetation. Each soil core was divided into two depths (subdivisions: 0–5 cm and 5–15 cm).

2.6. *Soil chemical analyses*

To understand whether Si supplementation altered soil chemical properties at the two depths, soil pH measurements were conducted using a pH meter (Mettler-Toledo Ltd., Australia) on 0.01 M $CaCl_2$ (Sigma-Aldrich, Australia); this method is less influenced by soil electrolyte concentration and thus gives a more consistent result than measurements in water (Minasny et al., 2011). Concentrations of total soil C and N were also quantified using a LECO TruMac CN-analyser (LECO Corporation, USA) according to an automated dry combustion method (Dumas), burnt at 1300 °C. Prior to analysis, collected soils at the two depths were oven-dried at 40 °C for five days, sieved and finely ground. Approximately 200 mg of soil per sample was required to conduct the analysis. Soil C and N were expressed as concentrations in % of dry mass unit.

In addition to total soil C and N, Si supplementation might affect the bioavailability of N, such as ammonium and nitrate which are often limited in the upper soil horizons. Soil concentrations of bioavailable N and Si were quantified using KCl and $CaCl_2$ methods, respectively (Rayment et al., 2011) at the Environmental Analysis Laboratory (Southern Cross University, NSW). Prior to these analyses, all samples were oven dried at 40 °C, weighed, sieved and gently crushed to < 2 mm.

Si supplementation in the form of slag-based fertiliser may be leaching and releasing some trace elements deeper in the soil (5–15 cm), changing total concentrations of certain metal(-loids). Thus, soil samples were analysed for 14 elements (arsenic, beryllium, boron, cadmium,

Table 1
Full details of field sampling campaign comprising soil, plant and arthropod collections across sampling dates.

Properties	Response variables	Sampling dates 09-Oct-18	20-Nov-18	18-Dec-18	24-Jan-19	28-Feb-19	18-Apr-19	21-Jun-19	10-Oct-19	21-Nov-19	20-Dec-19	28-Jan-20	12-Mar-20	12-May-20
Soil (0–5 cm)	pH ($CaCl_2$)	✓					✓		✓					✓
	Total C and N	✓					✓		✓					✓
	Bioavailable Si and N	✓					✓		✓					✓
Soil (5–15 cm)	pH ($CaCl_2$)	✓					✓		✓					✓
	Total C and N	✓					✓		✓					✓
	Metals	✓					✓		✓					✓
Plant	Dry biomass	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Forage quality	✓			✓				✓					✓
	Si and Ca	✓			✓				✓					✓
	C and N	✓			✓				✓					✓
Arthropod	Abundance	✓				✓			✓					✓
	Diversity	✓				✓			✓					✓

chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium, vanadium and zinc) at Eurofins Australia. Approximately 500 g of soil was provided to a NATA accredited third party laboratory for total solids analysis by Inductively Coupled Plasma (ICP) - Atomic Emission Spectrometry (AES) (USEPA SW-846 Method 6010 C) and leachability analysis by Toxicity Characteristic Leaching Procedure (USEPA SW-846 Method 1311). This is in accordance with the National Environment Protection (Assessment of Site Contamination) Measure, April 2011, Schedule B3 as per the requirements of the New South Wales Environment Protection Authority Steel Furnace Slag Resource Recovery Order 2017 and The ASMS steel furnace slag trial exemption 2018.

2.7. Plant chemical analyses

Si supplementation might alter Si and Ca accumulation in legumes. To investigate this, dried lucerne tissue was finely ground using a ball-mill. Approximately 80 mg of ground foliar tissue were subsequently analysed for total Si and Ca concentrations with an X-ray fluorescence spectrometer (Epsilon-3x, PANalytical-Almelo, the Netherlands) using a method similar to that of Reidinger et al., (2012). Analysis was calibrated using plant material (NCS ZC73018 Citrus leaves, China National Institute for Iron and Steel) of known Si concentrations (Hiltpold et al., 2017). Percentage (%) dry mass and mg kg^{-1} were used as units for foliar Si and Ca, respectively.

Besides Si and Ca, Si supplementation may potentially influence foliar concentrations of C and N. Foliar samples were finely ground using a ball-miller and packed (6–7 mg) in tin capsules for foliar C and N analyses using an Elementar-Vario EL Cube Analyser (Elementar Analysensysteme GmbH, Hanau, Germany) based on an automated dry combustion method (Dumas) and burnt at 950 °C. Concentrations in % of dry mass were used as the unit for foliar C and N.

2.8. Forage nutritional quality

Dried lucerne tissue was analysed for forage quality at Forage Lab Australia using the NIR (Near Infrared Spectrophotometry). Prior to this, dried tissue was ground to 1 mm and scanned using an infrared light source. The refraction of the light was then compared to a database for each constituent (Cumberland Valley Analytical, USA). We focused on 11 important parameters: neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein and fat, non-fibre carbohydrates, ash, lignin, proximate of hemicellulose, proximate of cellulose, total digestible nutrient and relative feed value (see glossary in the supplementary information).

2.9. Community responses of aboveground arthropods

Field supplementation of Si may indirectly affect aboveground arthropod communities via plant-mediated effects. Hence, diurnally active arthropods were collected using a suction technique with a vacuum device known as a petrol-powered ‘G-Vac’ (SH 86 C; Stihl AG and Co. KG, Germany) (Facey et al., 2017). Arthropods were vacuumed into an organza bag in a zig-zag method for 30 s over the plot in the sampling zone (1 × 2 m) and collected four times (Table 1). Debris (non--arthropods materials) was removed from bags, then, arthropod samples were stored in the freezer (−20 °C) on the same day for further analyses.

To determine the impacts of Si on abundance, diversity and functioning of arthropod communities, we first counted individual arthropods and grouped them morphometrically. Further identification of the individuals at the Order and family levels was determined using dichotomous keys, based on some main morphological features, such as wing, mouthpart, antennae and leg patterns. We validated our identification with various arthropod taxonomical textbooks and online sources especially for arthropods associated with lucerne in New South Wales (Bishop and Holtkamp, 1982; Pearce and Zalucki, 2005). We also classified the identified individuals at the family level for their potential functional roles in the terrestrial ecosystem (Facey et al., 2017): herbivores, predators, parasitoids and saprophytes. Due to a very low number of honeybees (Apidae) and pollinating flies (Diptera) from the collections, hence potential pollinators were excluded. We used taxonomical (Order and family) and functional levels to calculate Shannon diversity index, which accounts for species richness and evenness (Aguilera et al., 2020).

2.10. Statistical analyses

All statistical analyses were performed in R version 4.0.5 (R Core Team, 2021). To understand the effects of silicon supplementation, sampling dates and their interactions on soil and plant responses, we employed linear mixed-effects models by considering plots as the random effect to account for the non-independence of repeated measures using the ‘lmer’ function from the ‘lme4’ package (Bates et al., 2015). Soil chemical responses were separately analysed based on their soil depths (0–5 cm and 5–15 cm). Generated models were assessed to fulfil the assumptions of normality and the homogeneity of variances using the ‘qqPlot’ and ‘residualPlot’ functions from the ‘car’ package (Fox and Weisberg, 2019), respectively. Data were either square-root or $\log_e$ transformed if necessary. When significant effects ($p < 0.05$) of Si supplementation and or interaction with sampling date were observed, Tukey’s HSD post-hoc tests were performed to estimate differences among Si treatment levels ([-Si vs +Si], [-Si vs ++Si] and [+Si vs ++Si]) using the ‘pairs’ and ‘cld’ functions from the ‘multcomp’ package (Hothorn et al., 2021). We did not perform statistical analyses on soil elements that were below the detection limit (i.e. beryllium, boron, cadmium, mercury, molybdenum and selenium). Vanadium (V) was only detected in May 2020 and thus was still included in the analysis on that date.

To examine whether there were significant relationships between certain dependent variables, such as soil pH vs bioavailable soil Si, and shoot dry biomass vs foliar concentrations of Si, we used multiple regression models using the 'lm' function from the 'stats' package (R Core Team, 2021).

To investigate the indirect impacts of Si supplementation on aboveground arthropod responses, counts of individuals across plots and sampling dates were used to assess the arthropod abundance and diversity. We excluded some specimens which were not well preserved or poorly represented (fewer than 3% of the total number of individuals). To visualise the community composition of arthropods (i.e. abundance) in response to Si supplementation, non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity matrices were produced using the 'metaMDS' function from the 'vegan' package (Oksanen et al., 2020). Subsequently, the emerging patterns from NMDS plots were assessed with multivariate generalised linear models and a negative binomial error distribution using the 'manyglm' function from the 'mvabund' package (Wang et al., 2021). Then, to analyse responses of specific groups, we computed univariate analyses derived from the associated multivariate models for abundance on individual taxonomical members and functional groups using the 'anova' function from the 'mvabund' package (Wang et al., 2021). Total number of identified individuals was analysed using the 'glmer.nb' function and a negative binomial GLMM from the 'lme4' package (Bates et al., 2015). Analysis of deviance of the GLMM model was applied to confirm statistical significance using the 'Anova' function from the 'car' package (Fox and Weisberg, 2019). To have a better understanding of indirect Si impacts on the arthropod diversity at the Order, family and functional group levels, we calculated the Shannon diversity index using the 'diversity' function from the 'vegan' package (Oksanen et al., 2020). Finally, these indexes were analysed with linear mixed-effects as described above to assess the extent to which Si supplementation indirectly affected the diversity of identified arthropods at the taxonomical as well as the functional levels via plant-mediated effects.

3. Results

3.1. Si supplementation increased bioavailable Si and soil pH (0–5 cm depths)

Si supplementation caused consistent increases in bioavailability of soil Si with magnitudes increasing with application rates, across the different sampling dates (Fig. 2a, Table 2). For example, the bioavailability of soil Si increased up to 104% in +Si plots and 181% in ++Si plots, relative to -Si plots. There were significant increases of soil bioavailable Si up to 38% between +Si and ++Si plots.

Si supplementation increased soil pH at soil depths of 0–5 cm (Fig. 2b), but not that of 5–15 cm (Table 2). Increased soil pH (0–5 cm), however, depended on sampling date (Fig. 2b, Table 2). For example, Si supplementation increased soil pH in the first year (Oct 2018 and Apr 2019) but did not impact soil pH in the second year (Oct 2019 and May 2020). Relative to untreated plots, soil pH increased by 15% and 21% in +Si and ++Si plots, respectively in the first year. Increased soil pH was associated with increased bioavailability of soil Si, except in Oct-2019 (Fig. 2c, Table 2).

3.2. Si supplementation promoted crop yield but had a marginally significant impact on foliar concentrations of Si

Regardless of Si treatments, the average yield varied with season, which was the greatest during Australian summer (December – February) (Fig. 3a, Table 3). Si supplementation increased yield in Nov and Dec 2018, Oct and Dec 2019, and Jan and Mar 2020 (Fig. 3a, Table 3). Relative to -Si plots, Si supplementation increased lucerne yield on average by 31.5% and the degree of increase did not depend on

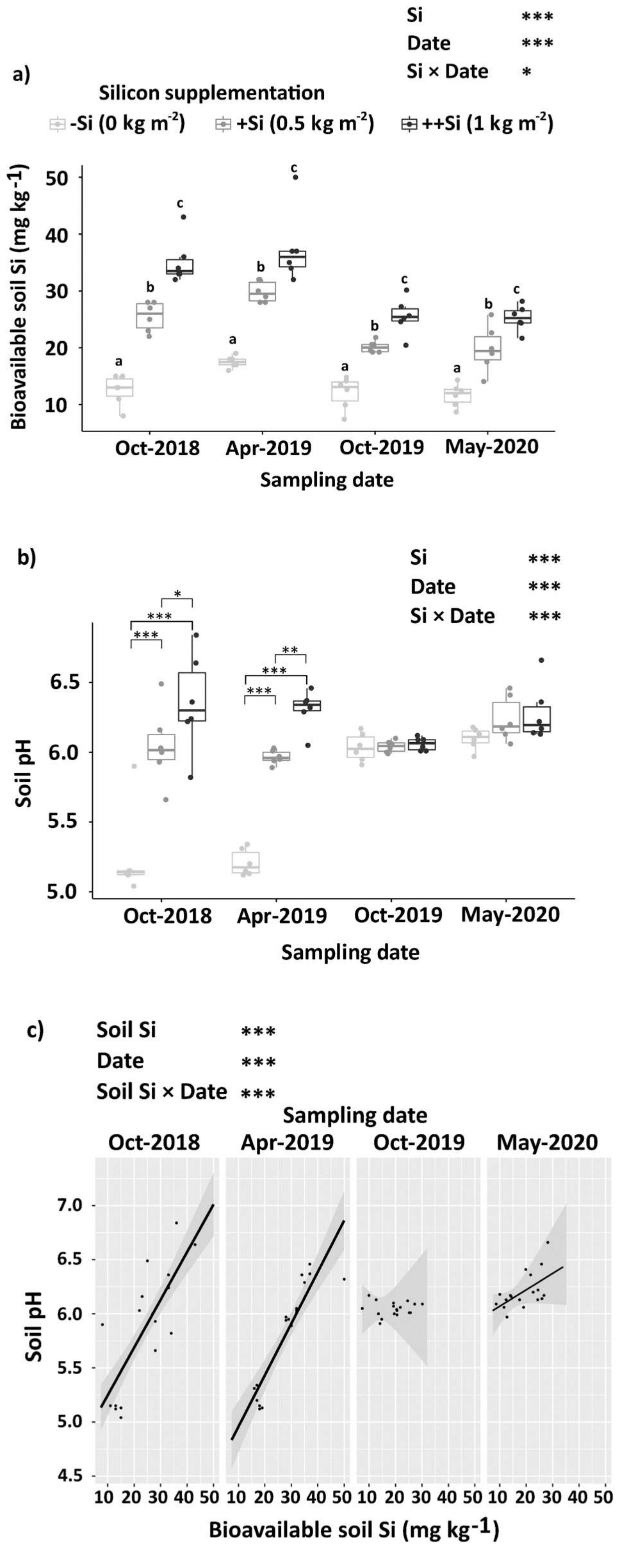


Fig. 2. The effects of Si supplementation (-Si, +Si and ++Si) on: a) bioavailable soil Si (mg kg^{-1}) and b) soil pH at depths 0 – 5 cm across sampling dates, and c) multiple linear regression models with a continuous predictor (bioavailable soil Si), a factor (sampling date) and their interactions on a criterion (soil pH). Dots represent individual data points ($n = 6$ per Si supplementation treatment), each regression line represents a slope of the model (predicted effect) for each factor and each ribbon defines 95% CI. Statistically significant factors are shown as: $^{*}p < 0.05$ and $^{***}p < 0.001$. Different letters (a – c) or asterisks (*) above the plots indicate statistical significance at $p < 0.05$ between Si supplementation treatment based on contrast tests.

Table 2
Summary of the effects of Si, date and their interactions on soil responses using linear mixed-effects models and the relationship between soil pH and soil bioavailable Si using multiple regression linear model. *p*-values shown in bold indicate significance ($p < 0.05$).

Linear mixed-effects	Factors					
	Si		Date		Si × Date	
Response variables	F	*p*	F	*p*	F	*p*
Depths (0–5 cm)						
Bioavailable Si	182.906	< **0.001**	35.859	< **0.001**	3.246	**0.010**
Soil pH	61.268	< **0.001**	15.217	< **0.001**	15.463	< **0.001**
Soil C	1.217	0.360	2.740	0.073	1.183	0.358
Soil N	1.990	0.217	2.931	0.061	1.080	0.410
Soil C/N	0.260	0.779	1.797	0.184	1.275	0.317
Ammonium	2.092	0.158	11.889	< **0.001**	0.948	0.471
Nitrate	1.303	0.301	91.722	< **0.001**	1.444	0.219
Depths (5–15 cm)						
Soil pH	1.239	0.318	111.417	< **0.001**	1.792	0.122
Soil C	1.866	0.234	4.731	**0.013**	0.745	0.621
Soil N	1.990	0.217	2.931	0.061	1.080	0.410
Soil C/N	0.513	0.623	2.204	0.123	0.580	0.742
($\log_e$) Arsenic (As)	0.715	0.505	20.829	< **0.001**	2.353	**0.046**
Chromium (Cr)	1.740	0.209	2.997	**0.040**	1.278	0.286
($\log_e$) Copper (Cu)	1.072	0.367	13.173	< **0.001**	2.290	0.052
Lead (Pb)	0.581	0.571	14.637	< **0.001**	1.597	0.170
($\log_e$) Manganese (Mn)	0.784	0.474	9.684	< **0.001**	2.866	**0.019**
Nickel (Ni)	0.578	0.573	15.282	< **0.001**	1.464	0.212
($\log_e$) Zinc (Zn)	0.362	0.702	10.950	< **0.001**	2.154	0.065
Vanadium (V)	0.092	0.913				
Multiple regression	Predictors					
	Soil Si		Date		Soil Si × Date	
Response variable	F	*p*	F	*p*	F	*p*
Soil (0–5 cm) pH	108.356	< **0.001**	30.138	< **0.001**	9.572	< **0.001**

Si application rate.

Foliar concentrations of Si were marginally significantly affected by Si supplementation at the 95% confidence interval ($p = 0.054$, Fig. 3b, Table 3). Contrast tests showed that there was a significant difference between -Si and ++Si supplementation ($p = 0.045$; Fig. 3b) with the highest increase at +126% in Oct 2019. Finally, there was a trend for foliar concentrations of Si to be positively correlated with yield ($p = 0.084$, Table 3).

3.3. *Si supplementation altered arthropod communities*

Collected 13,640 individuals were identified and classified into their corresponding Order (Table S1a). Identification to the family level resulted in 13,261 individuals due to ambiguous identification of 379 rarer individuals (Table S1b) and the individuals at the family level were further classified into four functional groups, namely herbivore, predator, parasitoid and saprophyte. At the functional level, some family members (i.e. Calliphoridae, Miridae and Muscidae) might occupy more than one functional role. Therefore, total individuals at the Order level (i.e. 13,640) were used to represent the total abundance of arthropods collected in this study. The list of classified arthropod individuals can be found in the supplementary information (Table S6).

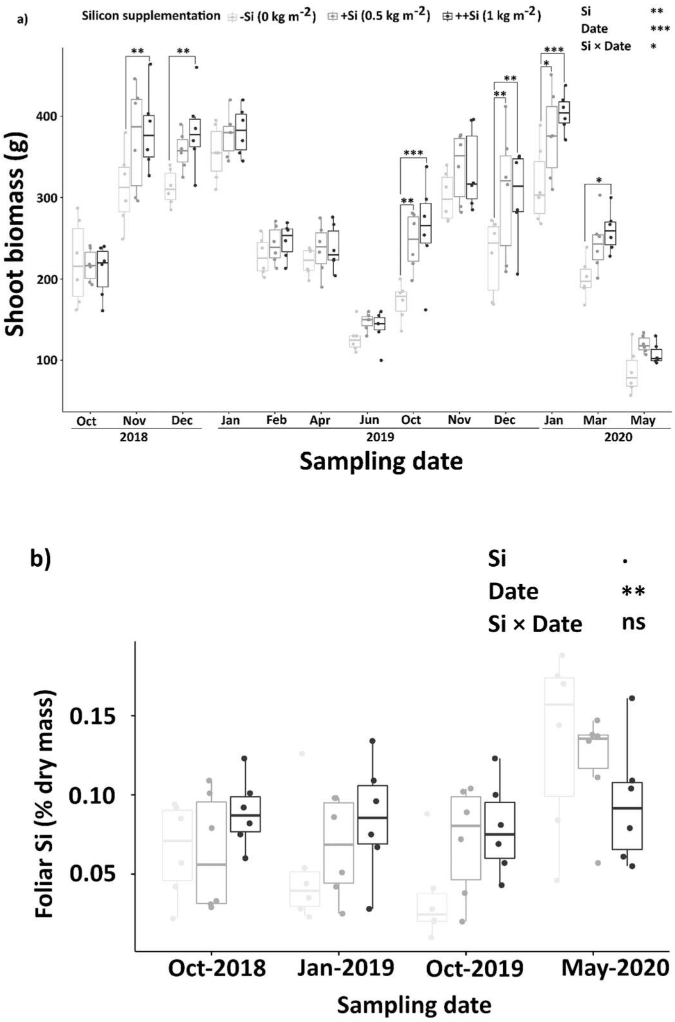


Fig. 3. The effects of Si supplementation (-Si, +Si and ++Si) on: a) shoot dry biomass (g) and b) foliar Si (% dry mass) across sampling dates. Dots represent individual data points ($n = 6$ per Si supplementation treatment). Statistically significant factors are shown as: ns (non-significant), $^{\bullet}p = 0.05 - 0.059$ (marginally non-significant), $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. Asterisks (*) above the plots indicate statistical significance at $p < 0.05$ between Si supplementation treatment based on contrast tests.

The NMDS plots showed that sampling date distinguished the community composition of arthropods at their taxonomical (Order and family) and functional levels more clearly than Si supplementation (Fig. S1a, b and c). Si supplementation did not alter total abundance of arthropods at the Order and functional levels, but, they both varied with sampling date (Table 4, Table S5g, Table S5i). However, at the family level, interactions between Si and sampling date were observed (Table 4, Table S5h). More specifically, univariate analyses at the family level showed that Lauxaniidae had clear increases in abundance with Si supplementation on the first three sampling dates relative to -Si plots (Fig. 4a). In addition, we also found significant interactive effects between Si and sampling date on total abundance of some family members, such as Agromyzidae, Sciaridae, Lygaeidae and Hemerobiidae (Table S3). However, those family members were found less frequently across group treatments and hence complicated the interpretation of responses. For example, Si tended to increase total abundance of Sciaridae in comparison to -Si plots, but it was only found more predominantly in Oct 2019 and May 2020 (Table S3). The summary of univariate analyses on the total abundance of the arthropod is provided in Table S2, S3 and S4.

Si alone did not significantly alter Shannon diversity at the Order and

Table 3
Summary of the effects of Si, date and their interactions on plant traits (biomass, elements and nutritional quality) using linear mixed-effects models and the relationship between shoot biomass and foliar concentrations of Si using multiple regression linear model. *p*-values shown in bold indicate significance ($p < 0.05$).

Linear mixed-effects	Factors					
	Si		Date		Si × Date	
Response variables	F	*p*	F	*p*	F	*p*
Shoot dry biomass	9.514	**0.002**	116.576	< **0.001**	1.615	**0.042**
($\log_e$) Foliar Si	3.564	0.054	6.810	**0.001**	1.914	0.099
Foliar Ca	1.494	0.256	10.964	< **0.001**	0.415	0.865
Foliar C	1.506	0.253	2.384	0.082	1.887	0.104
Foliar N	0.176	0.840	62.770	< **0.001**	0.825	0.556
Foliar C/N	0.748	0.490	55.027	< **0.001**	0.721	0.635
NDF	0.745	0.491	6.090	**0.001**	0.508	0.799
ADF	0.920	0.420	2.371	0.083	0.536	0.778
Lignin	0.898	0.428	33.225	< **0.001**	0.385	0.884
Hemicellulose	0.680	0.521	53.285	< **0.001**	1.228	0.310
Cellulose	0.940	0.412	0.276	0.842	0.587	0.739
Ash	0.370	0.697	4.179	**0.011**	0.710	0.643
Protein	0.241	0.789	12.932	< **0.001**	0.480	0.819
Fat	1.077	0.366	56.822	< **0.001**	1.498	0.200
Non-fibre carbohydrate	0.694	0.515	44.261	< **0.001**	0.934	0.480
Digested nutrient	0.773	0.479	28.582	< **0.001**	0.629	0.706
Feed value	0.983	0.397	4.075	**0.012**	0.498	0.806
Multiple regression	Predictors					
	($\log_e$) Foliar Si		Date		($\log_e$) Foliar Si × Date	
Response variable	F	*p*	F	*p*	F	*p*
Shoot biomass	3.089	0.084	143.471	< **0.001**	1.112	0.351

Table 4
Summary of the effects of Si, date and their interactions on the global patterns of abundance of the arthropods (Order, family and functional levels) using multivariate analyses, the total individuals using generalised linear mixed-effects, and the Shannon diversity index of the arthropods using linear mixed-effects models. *p*-values shown in bold indicate significance ($p < 0.05$).

Multivariate	Factors					
	Si		Date		Si × Date	
Response variables	Dev	*p*	Dev	*p*	Dev	*p*
Order	13.100	0.834	360.600	< **0.001**	70.100	0.104
Family	44.900	0.548	880.500	< **0.001**	203.100	**0.017**
Functional	9.15	0.343	153.95	< **0.001**	19.84	0.819
Generalised linear mixed-effects model	Factors Si		Date		Si × Date	
Response variable	X^2	*p*	X^2	*p*	X^2	*p*
Total individuals	0.541	0.763	82.477	< **0.001**	9.750	0.136
Linear mixed-effects model	Factors					
Shannon diversity	Si		Date		Si × Date	
Response variables	F	*p*	F	*p*	F	*p*
Order	0.764	0.483	1.601	0.202	0.464	0.831
Family	2.131	0.153	17.477	< **0.001**	2.198	0.061
(sqrt) Functional	3.944	**0.042**	9.428	< **0.001**	0.794	0.5798
(sqrt) Herbivore	0.686	0.519	17.702	< **0.001**	2.211	0.059
(sqrt) Predator	0.731	0.498	6.805	< **0.001**	2.459	**0.040**
(sqrt) Parasitoid	0.277	0.762	4.105	**0.012**	0.721	0.635
(sqrt) Saprophyte	3.594	0.053	11.788	< **0.001**	1.805	0.120

the family levels (Table 4). However, Si indirectly increased Shannon diversity index at the overall functional level (Fig. 4b, Table 4), e.g. the diversity of predators especially in May 2020 (Fig. 4d). The other patterns on the diversity of herbivores and saprophytes are also displayed (Fig. 4c, e).

3.4. Si supplementation did not influence soil elemental concentrations or forage quality

Si supplementation had negligible effects on total soil carbon and nitrogen in both depths, bioavailable ammonium and nitrate (0–5 cm depths) as well as the total concentrations of trace elements (5–15 cm depths) (Table 2). In terms of soil trace elements, only eight metal(-loid)s were detected, such as arsenic, chromium, copper, lead, manganese, nickel, zinc and vanadium (in May 2020 only) and the rest were below detection limits (i.e. beryllium, boron, cadmium, mercury, molybdenum and selenium). We found significant interactive effects between Si and sampling date only for arsenic and manganese, specifically concentrations decreased in ++Si plots relative to -Si ($p = 0.015$) and +Si ($p = 0.041$) plots in Oct 2019, based on contrast tests (Table 2). The levels of decrease, however, were low and this might be batch effects rather than the effect of sampling date because small differences between batches would probably result in significant differences. Furthermore, toxic heavy metals, for instance, chromium and lead were not affected by Si supplementation (Table 2).

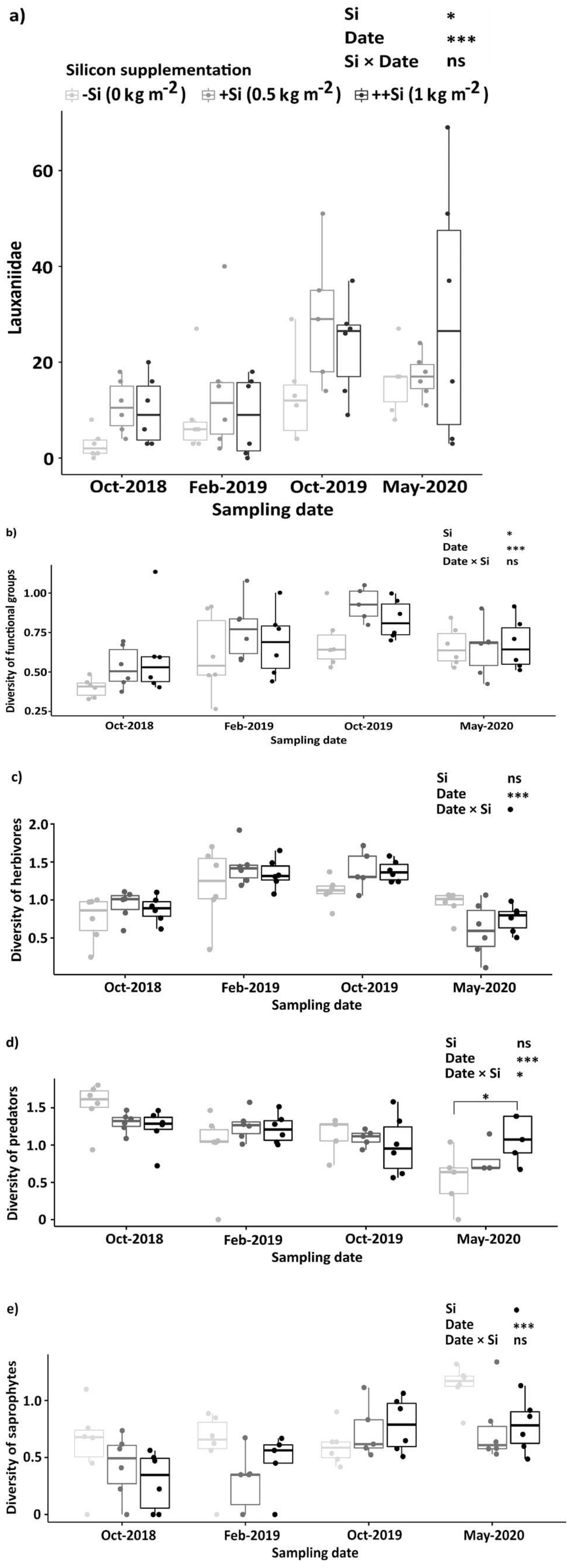
Si supplementation had no impacts on forage quality (Table 3), such as leaf carbon, nitrogen, calcium and the other 11 plant nutritional indices measured. Measures of crop digestibility, such as NDF and ADF, were also not altered by Si (Table 3). While the most important findings are reported here, the mean ± SD values of the response variables are provided in Table S5a – Table S5j.

4. Discussion

This study provides novel evidence that field application of Si can be beneficial for lucerne. Field supplementation of Si resulted in substantial increases in yield during a two-year experiment without reducing forage quality, as well as increasing soil pH to levels that are more favourable for growth of lucerne. We found that moderate Si supplementation (5 tonne ha^{-1} or 0.5 kg m^{-2}) was sufficient to produce these benefits. Moreover, Si supplementation caused indirect impacts on aboveground arthropods, including increased abundance of some fly communities, overall functional diversity and the diversity of predators. Contrary to our hypothesis about potential leaching of Si-slag complex, Si supplementation did not significantly increase soil toxic heavy metals.

4.1. Si increased crop yield without affecting plant elemental chemistry and forage nutritional quality

We showed that Si supplementation led to significant increases in lucerne yield relative to untreated plots. Previous glasshouse studies also found that Si supplementation increased the aboveground biomass of lucerne (Johnson et al., 2018a, 2017; Putra et al., 2021; Ryalls et al., 2023). The mechanism underpinning this benefit could be facilitated by the increased soil pH in this present study, which was more favourable for growth. However, Putra et al., 2021 demonstrated that increased



(caption on next column)

Fig. 4. The effects of Si supplementation (-Si, +Si and ++Si) on: a) total abundance of Lauxaniidae flies (Order: Diptera), b) Shannon diversity index of arthropods at the overall functional level and of c) herbivores, d) predators and e) saprophytes across sampling dates. Dots represent individual data points ($n = 4 – 6$ per Si supplementation treatment). Statistically significant factors are shown as: ns (non-significant), $^{\bullet}p = 0.05 – 0.059$ (marginally non-significant), $^{*}p < 0.05$ and $^{***}p < 0.001$. An asterisk (*) above the plots indicates statistical significance at $p < 0.05$ between Si supplementation treatment based on contrast tests.

shoot biomass of lucerne was positively associated with increased silicification in the foliage under controlled glasshouse conditions. Silicification might enhance physical resistance traits and rigidity of cell walls which has been observed in non-legumes, such as rice, maize and sugarcane (Ma and Yamaji, 2006). Another possible mechanism is that Si supplementation could increase water availability in soil, as reported in Schaller et al., 2023 in wheat fields.

Under field circumstances, it is likely that plants are subjected to a range of biotic and abiotic stresses, and thus Si-supplemented plants might be able to perform better as silicification often alleviates stresses relative to un-supplemented plants (Cooke and Leishman, 2016; Reynolds et al., 2016). However, similar to all open-field experiments, seasonal and climatic effects often contribute to variation in crop yield. For example, extreme occurrences of heat and winds in 2019 and 2020 damaged some of our lucerne crops at the field site where this experiment was conducted.

Our result indicates that Si supplementation marginally increased foliar concentrations of Si. It could be possible that the Si from slag must be released first over time and become available for lucerne. A longer time might also be needed for lucerne to accumulate Si in the foliage as it is a low-Si accumulator (Putra et al., 2021). In a recent field study, Johnson et al. (2022) argued that increased biomass as a result of Si supplementation might not necessarily translate into increased foliar concentrations of Si due to the dilution effect caused by rapid growth in Si-treated plants.

Si supplementation did not significantly affect foliar elemental chemistry of lucerne, such as C, N and Ca. Previous studies in grasses demonstrated a trade-off between Si and C (Neu et al., 2017; Klotzbücher et al., 2018; Biru et al., 2021), though this may be due to their very high uptake of Si that it can replace C both structurally and defensively. The relationship between Si and N is not clear-cut and might be species-specific. For example, an antagonistic relationship between Si and N occurred in rice (Wu et al., 2017) and in the grass species *Holcus lanatus* (Minden et al., 2021). Other studies demonstrated that Si addition increased root nodulation and N_2-fixation in symbiotic legumes associated with rhizobia (Nelwamondo and Dakora, 1999; Putra et al., 2021; Ryalls et al., 2023; Johnson et al., 2017), and thus Si and N could be positively correlated. A previous study reported that the application of calcium carbonate ($CaCO_3$), but not calcium silicate ($CaSiO_3$), increased foliar concentrations of Ca in Sudan grass (Monteith and Sherman, 1963). However, most legumes are high Ca accumulators (Schaller et al., 2017) and therefore, regardless of available Ca, legumes might have the capacity to maintain high shoot concentrations of Ca.

Forage quality is a crucial parameter for determining the potential risks associated with silicification in forage crops. Our findings here were in agreement with Van Soest and Jones (1968) that Si supplementation did not alter forage digestibility. Particularly, we show that Si-supplemented (+Si or ++Si) lucerne did not have different profiles of key forage digestibility indices, namely ADF, NDF, lignin and total digestibility nutrient relative to un-supplemented lucerne (-Si) (see the glossary in the supplementary information). In fact, *Medicago* legumes might not take up and accumulate higher concentrations of bioavailable Si in the shoots (Johnson et al., 2017; Putra et al., 2021; Ryalls et al., 2023) and therefore, it looks promising that these legumes could safely and effectively be used for animal feed in tandem with Si fertilisation.

4.2. Abundance of saprophytic fly communities and arthropod diversity at the functional level were associated with indirect effects of Si supplementation

The ecological role of Si is often studied in a simplified system, for example between a single plant species and an herbivorous insect (e.g. Johnson et al., 2020; Waterman et al., 2021) or between a plant and a pathogen (e.g. Fawe et al., 1998; Fauteux et al., 2006). However, the impacts of Si supplementation mediated by plants may extend beyond such bipartite interactions and affect the community (Hartley and DeGabriel, 2016), as plants interact with numerous arthropod communities (Johnson et al., 2009) and silicification is known to alter plant functional traits, such as growth, physiology and chemical composition (Cooke and Leishman, 2011a,b).

We showed, for the first time, that Si supplementation has indirect impacts on arthropod communities. In particular, a community shift favouring Lauxaniid flies to become more abundant was associated with Si supplementation in our system. Lauxaniid flies play an important role as saprophytes (Evenhuis and Okadome, 1989), potentially regulating nutrient cycling in the ecosystem (Johnson et al., 2018b). Adult Lauxaniids generally feed on fungi (Broadhead, 1984) and visit flowers (Kevan and Baker, 1983), whereas the larvae are typically saprophagous on fallen leaves (Miller and Foote, 1976). The production of leaf litter is predominantly regulated by plant productivity (Facelli and Pickett, 1991) and so, Si-enhanced aboveground plant biomass could increase lucerne turnover, resulting in more leaf litter and it was still digestible (or palatable), thereby indirectly promoting saprophytic fly abundance in Si-treated plots.

Si supplementation significantly increased arthropod diversity at the functional level, e.g. predators. Increased arthropod diversity could be mediated by changes in plant traits due to fertilisation (Johnson et al., 2018b; Evans and Sanderson, 2018). For example, increased crop productivity as a result of fertilisation may enhance insect diversity according to the 'resource rarity hypothesis' (Siemann, 1998), which suggests that increased plant biomass could provide refugia, food sources, compartmentalisation and distinct niches, supporting plant consumers and/or predators to coexist. This possible mechanism is worthy of further investigation.

Some arthropod individuals may occupy multiple niches, which can differ according to their life stages and even host plant developmental stages. For this reason, our current study proposes three suggestions: first, using multiple sampling methods may capture more arthropods from different niches, resulting in more functional groups (Facey et al., 2017); second, increasing the sampling frequency, for example, once a week before early bloom); and finally, confirming morphological taxonomy with the DNA metabarcoding method might help to gain a much deeper taxonomical resolution (Dopheide et al., 2020), thus identified individuals could then be assigned into their potential functional groups with a high degree of certainty.

4.3. Si supplementation affected pH and bioavailable Si but had negligible impacts on soil (toxic) trace elements

We found that Si supplementation in the form of blast-furnace slag consistently increased bioavailable Si in the soil across four different sampling periods. Our findings corroborate those of Haynes and Zhou (2018) who also showed that slag addition increased $CaCl_2$-extractable Si (mg kg^{-1}) in two different Australian soil profiles. The key mechanism here is the adsorption of silicate to soil colloid surface is pH specific (Hiemstra et al., 2007). Besides pH, Si availability is also governed by associated primary and secondary minerals (Haynes, 2014) and particle sizes (Rastogi et al., 2019). We utilised blast-furnace slag (mainly calcium silicate) that is considered a slow-release fertiliser which has a granular size less than 3 mm, and thus this might be more effective for a long-term application in perennial crops, such as lucerne. It is important to note that bioavailable Si presented in our study could also be partially contributed from the other minor biogenic Si pools in the soil, such as zoogenic, microbial and protistic Si (Haynes, 2017) or from phytogenic origins (Schaller et al., 2021).

We demonstrated that Si supplementation significantly increased soil pH. Increased soil pH was positively correlated with increased soil bioavailable Si. This is in line with previous studies (Haynes, 2014; Haynes and Zhou, 2018). The pH of blast-furnace slag itself is typically $>$ 10, releasing both silicic acid and OH^- ions during its dissolution and hence increasing soil pH to a greater extent, similar to an agricultural liming $CaCO_3$ (Haynes and Zhou, 2018). Si supplementation may be beneficial, particularly to ameliorate soil with high acidity ($pH < 5$) (Keeping et al., 2017), such as Chromosol (Isbell, 2016) in our study site. Acidic soils can be problematic for root nodulation and nitrogen fixation because they impair metal homeostasis and regulation of the rhizobial cells (Ferguson et al., 2013), thus applying this source of Si to increase soil pH may be particularly beneficial for symbiotic leguminous crops. However, whether field-Si application with slag becomes disadvantageous for plants that prefer acidic soils needs further investigations.

We observed negligible impacts of Si supplementation on the other soil elements, such as total C and N, bioavailable ammonium and nitrate, and the other eight elements, with the exception for As and Mn slightly decreased by Si. This might be a 'batch effect' because concentrations of both elements were a trace, thus even small differences between batches could generate this result. Elements contained in slags can vary depending on their materials, for example, calcium silicate and steel slag contain 35.5 and 213.6 mg kg^{-1} of As concentrations, respectively (Liang et al., 2015). It also remains unclear how long chemical constituents in slags will end up releasing into the soil and our study could not resolve this. However, Zhou and Haynes (2011) demonstrated that the application of steel slag and red mud increased some toxic elements, i.e. extractable As, Cr and Pb, but those were still below the regulatory limit (Haynes et al., 2013). Although negligible impacts were found here, other slags may have different chemical properties. Thus, an assay of a wider selection of materials and repeated slag application in the field should be tested to understand the possible risks of slag-based fertilisers for legumes.

5. Conclusion

Our two-year field experiment shows that Si supplementation in the form of blast-furnace slag fertiliser is beneficial for the productivity of lucerne and changing soil pH to be more beneficial to plants. Importantly, the application of Si in the field did not increase toxic elements in the soil or reduce forage nutritional quality. Si supplementation altered crop-associated arthropods, for example causing a shift towards saprophytic fly communities and increasing diversity of predators. Thus, our findings contribute to the ecological assessment of Si supplementation, particularly whether the application of Si-based fertilisers in the long term is an environmentally benign and sustainable method for increasing crop productivity and ecosystem functioning. For example, supplementation of slag fertilisers has been widely applied in Japan and the US (Liang et al., 2015; Tubana et al., 2016) without considering the implications for the wider ecosystem. Our findings with one slag source, at least, suggest that Si supplementation using industrial by-products could be a promising avenue for increasing bioavailable Si in the soil, thus increasing crop productivity.

CRediT authorship contribution statement

Putra Rocky: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Islam Tarikul:** Writing – review & editing, Data curation. **Cibils-Stewart Ximena:** Writing – review & editing, Data curation. **Hartley Susan (Sue) E.:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Johnson Scott N.:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.108893.

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