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RESEARCH ARTICLE

Frass pre-treatment and application method impact spring wheat vegetative growth and nutrient concentration

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Abstract

The house cricket (*Acheta domesticus*) is a widely farmed edible insect species, and its by-product – frass – has potential as a sustainable soil amendment. However, the efficacy of frass may depend on how it is pre-treated and applied. This study evaluated the effects of cricket frass on the vegetative growth and nutrient concentration of spring wheat (*Triticum aestivum*) under different frass pre-treatments and application methods. Wheat was pot-grown in a greenhouse for eight weeks — from seed germination to flowering — with one control (no frass) and six experimental treatments, combining two pre-treatments (non-heated and heat-treated frass) and three frass application methods (surface application, mixed into the substrate, and 2-week delayed application). Germination, plant height, shoot biomass, leaf chlorophyll content, and shoot nutrient concentration were measured. Frass pre-treatment and application method significantly influenced wheat growth and nutrient concentration. Germination reached 100% with heat-treated frass mixed into the substrate and delayed application of no-heat frass but dropped to 60% with surface-applied heat-treated frass. The tallest plants grew with no-heat frass mixed into the substrate, while surface application, regardless of heat treatment, produced the greatest shoot biomass. Delayed application, regardless of pre-treatment, resulted in the highest leaf chlorophyll content. Surface-applied heat-treated frass increased plant nitrogen and potassium concentrations, whereas no-heat mixed frass enhanced phosphorus levels. These findings demonstrate that cricket frass can improve wheat growth and nutrient concentration, but its effects vary by pre-treatment and application method. Optimising these factors could maximise its potential as a sustainable soil amendment in crop production.

Keywords

Acheta domesticus – biostimulant – edible insects – insect faeces – organic fertiliser

1 Introduction

The edible insect sector is expected to expand significantly within the coming years, offering an alternative solution to global food security challenges (Siddiqui *et al.*, 2023). However, alongside supplying food, the growth of the edible insect sector will result in an increased production of waste, such as frass. In the context of mass insect rearing, frass is a mixture of insect faeces, shed exoskeletons, dead insects, insect parts, and leftover feed. This mixture frequently contains an array of essential nutrients for plant growth

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that can be valuable as an organic fertiliser (Beesigamukama *et al.*, 2022). Frass can also be used as a biostimulant – a substance that enhances plant growth, nutrient acquisition, stress tolerance, and/or crop quality – due to the presence of chitin (a main component of the insect exoskeleton) and potentially beneficial microbes (Poveda *et al.*, 2019; Barragán-Fonseca *et al.*, 2022; Ferruzca-Campos *et al.*, 2023). The use of frass as a soil amendment, whether as a fertiliser or biostimulant, has therefore the potential to increase the sustainability of edible insect production by reducing the reliance on synthetic inputs in crop production and supporting the development of food system circularity (Smetana *et al.*, 2022).

Cereal crops, such as wheat (*Triticum aestivum*), are among the most widely cultivated staple crops globally, making them an important target for evaluating the agronomic potential of frass as a soil amendment. Several recent studies have investigated the potential of frass as a soil amendment, primarily testing frass sourced from black soldier fly larvae (*Hermetia illucens*) or mealworms (*Tenebrio molitor*). For example, black soldier fly frass applied at a rate of 7.5 t/ha increased maize grain yield by 27% compared with mineral fertilisers at equivalent nitrogen rates, highlighting the potential of frass to replace synthetic fertilisers (Beesigamukama *et al.*, 2020), and the same type of frass also increased vegetative shoot biomass and foliar nutrient content of barley, oats, spelt and triticale (Carroll *et al.*, 2023). Mealworm frass was also found to significantly improve barley vegetative biomass and nutrient uptake (Houben *et al.*, 2020). These findings suggest that frass may hold significant promise for improving plant growth and nutrient concentration in cereal crops.

However, while frass has shown benefits in certain growing conditions, the effectiveness of frass as a soil amendment can vary depending on several factors. For example, the type of insect that produced the frass and the composition of the frass (i.e. faeces or shed exoskeletons) can influence plant growth and rhizosphere communities (van de Zande *et al.*, 2024). Furthermore, the way frass is applied can affect the accessibility of nutrients to plants. While no studies have specifically compared frass application methods, a review and meta-analysis on fertiliser placement suggests that subsoil application of nutrients can enhance yield and nutrient uptake compared to surface broadcasting (Mehdi *et al.*, 2016). Additionally, heat pre-treatment of frass – commonly required for its use as fertiliser – may influence microbial composition, activity, and biomass (Praeg and Klammsteiner 2024).

As the edible insect sector grows, evaluating the effectiveness of frass as a soil amendment is crucial for understanding its agronomic potential. In this study, we investigated the use of frass from the house cricket (*Acheta domesticus*) as a soil amendment in spring wheat (*Triticum aestivum*) cultivation. *Acheta domesticus* is one of the most widely farmed insect species for food and feed due to the species' high protein content, efficient feed conversion, and rapid reproduction (Gahukar 2016; Pilco-Romero *et al.*, 2023). Despite its commercial significance, research on *Acheta domesticus* frass remains limited. The aim of this study was to evaluate the effects of *Acheta domesticus* frass on the vegetative growth and nutrient concentration of spring wheat, focusing on how these outcomes are influenced by different heat pre-treatments applied to the frass and by different frass application methods. We hypothesised that (H1) *Acheta domesticus* frass applied as fertiliser will enhance shoot biomass growth and nutrient concentration of young spring wheat plants; (H2) heat pre-treatment of the frass will impact the plant responses; and (H3) different frass application methods (surface application, mixed into the substrate, and 2-week delayed application) will influence the plant responses.

2 Materials and methods

Spring wheat (*Triticum aestivum*, var. 'Boett') was grown in a greenhouse for 8 weeks – from seed germination to flowering – at the Swedish University of Agricultural Sciences (Uppsala, Sweden) from September 10th to November 5th, 2023. During the growing period, the temperature in the greenhouse ranged between 22 and 25 °C and relative humidity between 28 and 61%. The greenhouse environment included artificial light conditions with a 16-hour light period and watering three times a week.

Four wheat seeds were planted directly into a 7.5-litre pot (d = 26 cm, h = 21 cm) at a depth of 2.5 cm, spaced 7.6 cm apart. Each pot had its own tray underneath to prevent nutrient leaching and cross contamination between frass treatments. For the growing medium, a low-nutrient potting mix (Supplementary Table S1) was used. The potting mix was composed of light and dark peat, perlite, sand/rock milk, lime, mineral fertiliser, and root powder, with a pH of 5.5–6.5.

TABLE 1 Nutrient content of the *Acheta domesticus* frass applied in the experiment, expressed in grams per kilogram of frass sample

Nutrients	Amount
Total nitrogen (N)	38.40
Phosphorous (P)	8.07
Potassium (K)	9.49
Calcium (Ca)	10.84
Magnesium (Mg)	4.71
Sodium (Na)	2.01
Sulphur (S)	4.58

Frass treatment and composition

Raw (untreated) frass was sourced from *Acheta domesticus* reared commercially as food for humans by an EU-based company, where the crickets were fed a grain-based diet, primarily consisting of barley, oats, and other plant-derived ingredients. The frass provided nitrogen, phosphorous, and potassium (NPK) in the proportions 4.7:1:1.2 (Table 1), which is similar to the corresponding proportions of 5.8:1:1.9 in many commercial NPK fertilisers. Before the experiment, all frass was stored at −10 °C for 2 days to kill viable eggs, cricket nymphs, and other insect pests that may be present. Frass was subsequently sieved (3 mm) to separate carcasses, large pieces of leftover feed, shed exoskeletons, and inorganic rearing material.

Experimental design

To evaluate the impact of application method, three common fertiliser application techniques – surface application, subsoil application, and side-dressing – were simulated to replicate standard agricultural practices (Jones, 2002). In the first method (henceforth *surface*), frass was applied to the surface of the pot after sowing, simulating surface broadcast fertilisation. In the second method (henceforth mix), frass was mixed into the substrate before sowing, simulating subsoil application, where fertiliser is incorporated below the surface using techniques such as ploughing, harrowing, or rotary tillage. In the third method (henceforth delay), frass was surface-applied two weeks after sowing, simulating side-dressing, where fertiliser is applied alongside crops after emergence.

To assess the effect of heat pre-treatment, each application method included two experimental groups. In the first group, non-heated frass (henceforth no-heat frass) was used, and in the second group heat-treated frass was used. The heat-treated frass was exposed to 70 °C for 1 h, following EU regulations on minimum processing requirements for animal by-products used as fertiliser (European Commission, 2021). The three application methods, each with frass either heat-treated or not, resulted in six experimental groups. In addition, a control group to which no frass was applied was included in the set-up.

For all seven treatment groups there were five replicates (i.e. pots). In each pot, four wheat seeds were planted in 2 kg of substrate (potting mix with or without frass). The potting mix provided 0.94 g of available nitrogen. To all groups except the control, 150 g of frass was added, corresponding to 7% (w/w) of the total substrate. This addition increased the total nitrogen availability by 5.76 g to a total of 6.70 g per pot (calculated as N concentration of frass (%) × frass amount (g)/100). A randomised design was implemented, with pot positions in the greenhouse rotated weekly to minimise potential side effects from light and temperature variations.

Measured outcomes

To assess how *Acheta domesticus* frass affects wheat growth – and how heat pre-treatment and application method influence vegetative growth and nutrient concentration – we measured germination, shoot height, and dry shoot biomass. Germination was measured as the percentage of wheat seeds that germinated out of the total number of seeds sown (i.e. the number of seeds germinated out of 20 seeds planted per treatment). Plant height was measured from the base of the stem to the highest node (the point on the stem where leaves emerge) using a hand-held ruler for each wheat seedling at week 8. To measure dry shoot biomass, wheat shoots from all plants were harvested at week 8, dried in an oven at 70 °C for 48 h, and weighed using a precision balance (ML1602T/00, Mettler-Toledo, Greifensee, Switzerland). Week 8 was selected as the endpoint for vegetative measurements because it coincided with the initial signs of reproductive development in the control group (i.e. seed head development), while all frass-treated plants remained in the vegetative stage, ensuring consistency in developmental phase across treatments.

To assess differences in plant nutrient concentration, leaf chlorophyll content and shoot nutrient concentration were measured. Leaf chlorophyll content, as assessed by SPAD, is commonly closely correlated with leaf nitrogen content (Uddling *et al.*, 2007). Therefore, leaf chlorophyll content was measured non-destructively using a SPAD chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Japan), averaging three readings taken from the middle of the uppermost leaf of each seedling before harvest (week 8). The total nitrogen (N) concentration of shoot biomass was determined using the Kjeldahl method with a 2520 Digestor, Kjeltec 8400 Analyser and 8460 Sampler (FOSS Analytical, Hillerød, Denmark). The concentration of phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), and calcium (Ca) in shoot biomass was analysed using a spectrophotometer (ICP-AES, ICP Spectro Flame, Spectro Analytical Instruments).

Statistical analysis

The data were analysed using the statistical software Stata version 14.2 (Stata, 2021). The unit of analysis throughout the study was the pot, with 5 pots per experimental group (and up to 20 plants per group). For the analyses of dry shoot biomass and shoot nutrient concentration, we used average measurements per plant within each pot to account for differences in germination, since variable plant numbers per pot would directly affect total biomass and nutrient values. This approach ensures that treatment effects reflect differences in plant performance, not just differences in germination. To complement the per-plant analysis, we also analysed total shoot biomass per pot to assess treatment effects at the pot level (Supplementary Figure S1).

Before analyses, the data were assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene tests, respectively. Normally distributed data were analysed using a one-way ANOVA, followed by Tukey's honest significant difference (HSD) test for pairwise comparisons when significant differences were identified ($p < 0.05$). For data that did not meet normality assumptions, the non-parametric Kruskal-Wallis test was used, with Dunn's test for pairwise comparisons when significant differences were observed ($p < 0.05$).

To compare the effects of frass and control treatments, a one-way ANOVA was applied. A two-way ANOVA was used to assess the impact of heat pre-treatment, application method, and their interaction. Since frass was not applied to control pots, the control group was excluded from the two-way ANOVA. Furthermore, a Pearson correlation analysis was used to evaluate the relationships between dry shoot biomass, height, and leaf chlorophyll content, using correlation coefficients (r) to quantify the strength and direction of the associations.

3 Results

The addition of *Acheta domesticus* frass as a soil amendment influenced multiple aspects of spring wheat growth. Furthermore, germination, plant height, shoot biomass, and plant nutrient concentration were all influenced by both heat pre-treatment and frass application method (Figure 1).

Germination

Heat pre-treatment affected germination differently depending on frass application method ($p = 0.023$) (Supplementary Tables S2–S4). Wheat that received heat-treated mixed application of frass and no-heat delayed application exhibited the highest percentage of germination (100 ± 0%), while heat-treated surface applied frass resulted in significantly reduced germination of the seeds (60 ± 13.7%) compared to all other treatments (Supplementary Tables S2–S4). No significant differences in germination were observed between the control and no-heat frass groups (Figure 1A, Supplementary Tables S2–S4); however, plants in the control group had significantly higher germination than plants with heat-treated, surface-applied frass ($p < 0.001$).

Height

Height of seedlings was significantly affected by both heat pre-treatment ($p = 0.013$) and application method ($p < 0.001$) of the frass (Supplementary Table S5–S7). Plants grown with no-heat frass were taller (27.05 ± 0.78 cm) than plants that received heat-treated frass (24.12 ± 0.78 cm). Wheat grown with no-heat frass mixed into the substrate before sowing produced the tallest plants (29.50 ± 1.31 cm), while surface application of heat-treated frass resulted in the shortest plants (20.48 ± 0.48 cm) (Figure 1B). No significant differences in plant height were observed between the control and no-heat frass groups; however, plants in the control group were significantly taller than those receiving heat-treated, surface-applied frass ($p = 0.001$).

Shoot biomass

Shoot biomass was significantly influenced by frass application method ($p = 0.004$), with surface application producing the highest biomass per plant across all treatments (5.82 ± 1.21 g) (Figure 1C, Supplementary Tables S8–S10). Neither pre-treatment of the frass, nor the interaction between pre-treatment and application method had any significant effects on the shoot biomass produced by the plants (Figure 1C). Furthermore, plants in the control group produced significantly

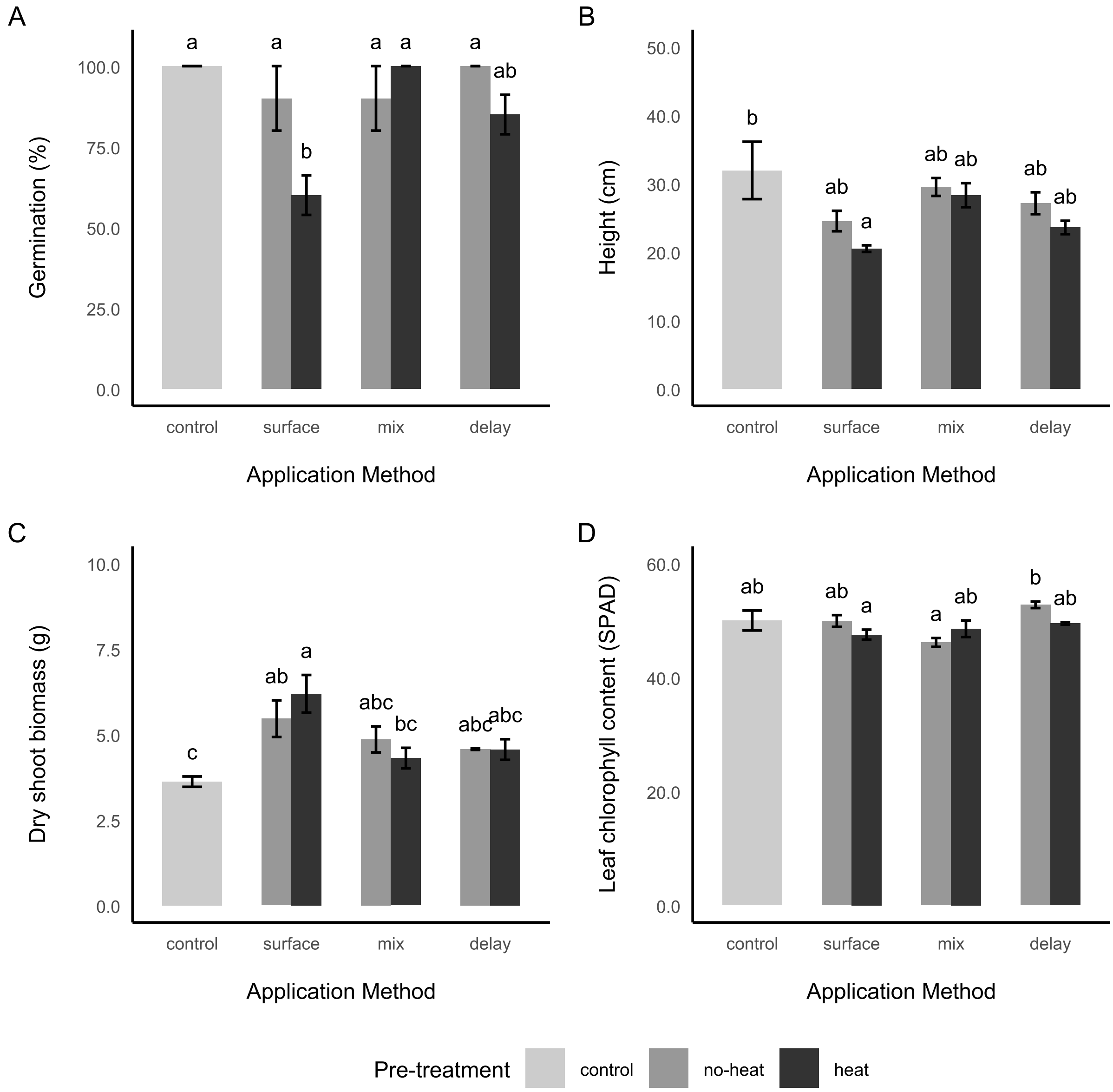

FIGURE 1 (A) seed germination (%), (B) plant height (cm), (C) dry shoot biomass (g) and (D) leaf chlorophyll content (SPAD) of young spring wheat plants pot-grown in a greenhouse at week 8 across treatments with no frass added (control), frass that is untreated (no-heat), and heat-treated frass (70 °C for 1 h) applied to the soil on the surface (surface), mixed into the soil before sowing (mix), or applied to the soil on the surface with a 2-week delay (delay). Each bar represents the mean of 5 pots per treatment, with error bars showing mean ± SE. Different lowercase letters above bars indicate statistically significant differences among treatments (Tukey HSD post-hoc test, $p < 0.05$).

less shoot biomass compared to those in surface-applied treatments, both with no-heat frass ($p = 0.007$) and heat-treated frass ($p = 0.001$) groups, but no significant differences were observed between the control and other application methods, regardless of the pre-treatment (Supplementary Tables S8–S10). A complementary analysis of total shoot biomass per pot showed a similar overall treatment effect ($p = 0.021$), though no pairwise differences were statistically significant (Supplementary Figure S1).

Leaf chlorophyll content and plant nutrient concentrations

The leaf chlorophyll content in the plants was affected by the frass application method ($p < 0.001$), and delayed application of frass, regardless of pre-treatment, resulted in the highest SPAD values (51.11 ± 1.95) compared to surface (48.70 ± 2.38) and mix (47.34 ± 2.76) applications (Figure 1D). The heat pre-treatment caused higher SPAD values compared with the no-heat pre-treatment only in the mixed application, but lower SPAD values in the other two application methods (Figure 1D), a pattern reflected by a significant interaction between heat treatment and application method (p = 0.01) (Supplementary Table S11). The pre-treatment alone had no significant effect (p = 0.155). Furthermore, no significant differences in leaf chlorophyll content were observed between the control and frass treatments, regardless of pre-treatment (Supplementary Table S11).

Shoot nutrient concentrations at week 8 varied significantly across treatments (Figure 2). Surface application of heat-treated frass resulted in the highest nitrogen and potassium concentrations. The concentration of phosphorus was highest in shoots from the no-heat mix application, while magnesium concentration peaked with surface-applied heat-treated frass. Sulphur and calcium concentrations were not significantly affected by either application method or heat treatment (Supplementary Table S6). Control treatments generally exhibited lower levels of shoot nutrients compared to frass-amended treatments, although the extent of these differences varied depending on the nutrient, heat-pre-treatment, and application method (Supplementary Table S12).

The correlation analysis revealed that shoot biomass was not significantly correlated with neither shoot height (p = 0.10) nor leaf chlorophyll content (p = 0.68). However, there was a significant positive correlation between shoot height and leaf chlorophyll content (p = 0.02, r = 0.36).

4 Discussion

This study shows that frass from *Acheta domesticus*, when added to the soil, can affect the growth and nutrient concentration of young wheat (*Triticum aestivum*) plants. Effects were seen in many plant traits measured and varied depending on if the frass was heat treated before use and how the frass was applied to the soil. Similar patterns have been observed with frass from other edible insect species, where mealworm frass enhanced barley biomass and nutrient cycling (Houben *et al.*, 2020), and black soldier fly larvae frass increased shoot biomass and foliage nutrient content in cereals (Carroll *et al.*, 2023). While our results partially support the hypothesis that *Acheta domesticus* frass would enhance shoot biomass growth and nutrient concentration of young spring wheat plants (H1), they also confirm that heat pre-treatment (H2) and application method (H3) significantly influence the plant responses. To our knowledge, no previous studies have specifically investigated how frass application methods influence crop performance. Research on conventional fertilisers have demonstrated that application techniques can significantly affect nutrient availability and plant growth (Mehdi *et al.*, 2016), implying that tailored frass application strategies could enhance agronomic benefits of frass. Additionally, while a heat pre-treatment of 70 °C for at least 1 h is essential for pathogen control, it can reduce microbial activity (Van Looveren *et al.*, 2022; Praeg and Klammsteiner 2024) and impact growing conditions. Together, these findings highlight the potential of insect frass as a sustainable soil amendment while emphasising the importance of optimising frass management and application protocols.

In our study, mixed application of heat-treated frass and delayed application of no-heat frass exhibited the highest germination, whereas surface application of heat-treated frass significantly reduced germination. One potential mechanism for the observed reduction in germination rates with surface-applied heat-treated frass is the combined effect of heat pre-treatment and application method, leading to chemical and biological alterations – e.g. the release of phytotoxic compounds (Cui *et al.*, 2024) or reduction of beneficial microbes – that may have altered soil conditions near the seed, with surface application potentially concentrating these effects and exposing seeds to unfavourable conditions. This study did not assess phytotoxicity nor microbial dynamics, which could provide further insight into the underlying mechanisms. Additionally, this result may be more pronounced in controlled greenhouse conditions using potting mix and may differ in a field setting, where greater variability in soil structure and microbial activity could mitigate negative effects.

Along with the reduced germination observed with surface-applied heat-treated frass, the plants generated by this heat pre-treatment exhibited the highest shoot biomass per plant. This pattern may be due to either reduced competition among fewer germinated seedlings (i.e. providing more nutrients per individual plant) and/or differences in shoot (tiller) number across treatments, as plants with fewer tillers tend to grow taller, while more tillers can result in shorter shoots but greater biomass. Additionally, application method played a critical role in determining plant height and shoot biomass, with mixing frass into the soil producing the tallest plants and surface application resulting in the greatest biomass per plant. In terms of nutrient concentration, delayed frass application, regardless of heat pre-treatment, produced the highest leaf chlorophyll content (SPAD) values, while shoot nutrient analysis revealed that surface-applied heat-treated frass led to

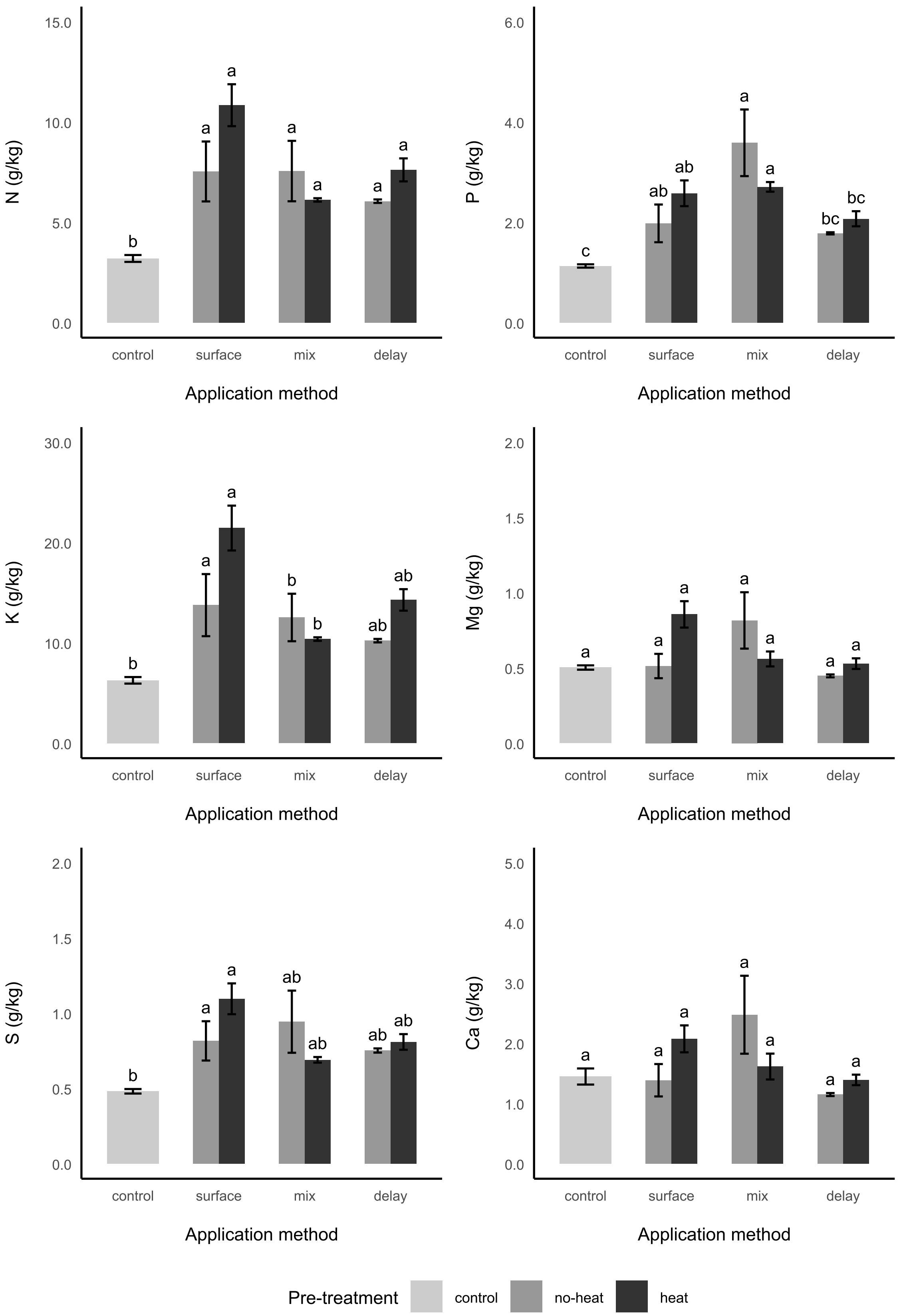

FIGURE 2 Nutrient concentrations (g/kg) of N, P, K, Mg and S in the shoots of 8-week-old spring wheat plants pot-grown in a greenhouse across treatments with no frass added (control), frass that is untreated (no-heat), and heat-treated frass (70 °C for 1 h) applied to the soil on the surface (surface), mixed into the soil before sowing (mix), or applied to the soil on the surface with a 2-week delay. Each bar represents the mean of 5 pots per treatment, with error bars showing mean ± SE. Different lowercase letters above bars indicate statistically significant differences among treatments (Dunn's post-hoc test, $p < 0.05$).

the highest shoot nitrogen and potassium concentrations.

An interesting finding of this study is the discrepancy between the effects of frass on height and biomass. While shoot biomass was not significantly correlated with height or leaf chlorophyll content (SPAD), which is in contrast to other studies on wheat (Hamnér *et al.*, 2017), plant height increased significantly with leaf chlorophyll content. This suggests that frass influenced shoot height and biomass in different ways, potentially affected by the number of plants (through germination rate), the number of tillers (shoots) produced per plant, and the specific wheat variety used. Moreover, vegetative growth may not necessarily translate into increased grain yield at harvest. While traits such as plant height and biomass are often used as proxies for productivity in controlled studies (Weiner, 2004), grain yield is determined by additional factors, such as the allocation of resources to reproductive structures during the grain-filling stage. Thus, under certain environmental conditions, greater plant biomass has even been reported to be associated with reduced yield (Thapa *et al.*, 2020). These dynamics can also differ between greenhouse and field settings, where nutrient availability, stressors, and competition are more variable (Asplund *et al.*, 2016). Future research should investigate how frass pre-treatments impact both vegetative growth and harvestable grain yield and quality under field conditions, addressing current gaps in understanding the direct effects of frass on crop productivity and grain quality.

Beyond the observed physiological effects on plant growth and nutrient concentrations, the practical feasibility of frass as a crop fertiliser must be considered. While frass demonstrates significant potential in crop production, today, its use as a fertiliser for large-scale field applications may be constrained by limited supply or high transaction costs, as the edible insect industry is still in its early stages. Frass may therefore be best used as a supplement to conventional fertilisers or as a targeted soil amendment (e.g. biostimulant) for specific stages of crop growth. Furthermore, while the use of a single crop species and one frass type limits the scope of our study, the simplified and controlled experimental design allowed us to clearly isolate treatment effects – an important step given the limited research on *Acheta domesticus* frass as a fertiliser. Future research should build upon these findings by evaluating different frass types across diverse cropping systems and investigating longer-term impacts of frass on soil health, nutrient cycling, and microbial communities in the field.

5 Conclusion

Overall, *Acheta domesticus* frass shows promise as a soil amendment for the vegetative growth and nutrient concentration of spring wheat. However, there are nuances to its use. Heat pre-treatment and application method are critical factors influencing the effectiveness of frass, with mixed and delayed applications improving germination, no-heat mixed application promoting greater plant height, and surface application of heat-treated frass increasing shoot biomass. In terms of nutrient concentrations, delayed application produced the highest leaf chlorophyll content and surface-applied heat-treated frass resulted in the highest shoot concentrations of nitrogen and potassium. By demonstrating how frass management and application strategies can impact plant growth and nutrient dynamics, this study contributes to the broader understanding of insect frass as a soil amendment.

Supplementary materials

Data is available on https://doi.org/10.1163/23524588-bja10245 under Supplementary Materials.

Conflict of interest

The authors declare no conflict of interest.

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