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The Effects of Insect Frass Fertilizer and Biochar on the Shoot Growth of Chicory and Plantain, Two Forage Herbs Commonly Used in Multispecies Swards

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Abstract: Livestock farmers are seeking ways to lower their environmental footprints by reducing inputs, lowering greenhouse gas emissions, and enhancing carbon sequestration. To meet these objectives, farmers are investigating the value of diverse multispecies grazing swards and various organic by-products as alternative fertilizers and soil amendments. This study investigated the effects of HexaFrass™ [HexaFly, Meath, Ireland], an insect frass based organic fertilizer, and biochar, a form of charcoal, on the shoot dry matter accumulation of two forage plants, chicory and ribwort plantain. Under glasshouse conditions, HexaFrass™ generally increased the shoot growth of both chicory and plantain, although these positive effects were lost if an excessive amount of HexaFrass™ was applied, or the growing medium was already nutrient-rich. Importantly, it was found that HexaFrass™ also increased the re-growth of shoots after cutting, which is vital for plants that are destined to be successfully used in grazing or silage swards. Biochar had a less obvious effect on the shoot dry matter accumulation, although there was some evidence of a synergy between biochar and HexaFrass™, which caused an additional increase in the shoot growth. The results indicate that frass-based fertilizers could play a role in low-input mixed swards, whereas the potential of biochar as a soil amendment in these grazing systems requires further research.

Keywords: black soldier fly; circular economy; grazing; organic farming; pasture; regenerative farming

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1. Introduction

In order to meet growing food demands and increase yields, dairy and livestock farmers have traditionally relied on expanding the area under cultivation and/or the application of high loads of synthetic fertilizers. These highly productive intensive farming systems are, however, under increasing scrutiny because of their association with negative environmental impacts and ecological damage [1]. The conversion of land to agricultural land can result in the loss of natural habitats and a decrease in local biodiversity. Additionally, the application of extreme quantities of nitrogen-based fertilizers is often associated with a suite of environmental issues, such as increased nutrient run-off, the pollution of water ways, and greater release of greenhouse gases [2–5]. In order to achieve more sustainable food production, farmers and legislators are now examining all aspects of farming systems, including the genetics and husbandry of livestock, expanding the range and diversity of plant species used in grazing swards, and exploring alternative soil amendments that can maintain plant growth while mitigating environmental footprints [2,6].

Increasingly, it is being realized that there can be synergy between agri-environment schemes in terms of efforts to simultaneously address multiple issues centered on the improvement of food production sustainability, reduction of environmental damage, and enhancement of farmland biodiversity [7]. For example, the fencing-off of farmland streams to prohibit access by livestock helps to stabilize banks, reduces water turbidity, and prevents the direct pollution of waterways with urine [8,9]. Revegetating these riparian spaces with native plant species provides additional benefits, such as nutrient trapping, carbon sequestration, and increased invertebrate diversity, which in turn can lead to enhanced ecosystem services, such as pollination and pest control [8,10].

One initiative that farmers are adopting in order to address multiple sustainability and environmental issues is the incorporation of multispecies swards (MSS), or herbal leys, into grazing systems. The inclusion of nitrogen-fixing legumes, such as clovers, and forage herbs, such as plantain and chicory, in MSS can help to lower the required inputs of synthetic fertilizers, reduce nutrient leaching, and enhance the forage nutritional quality [11–13]. The dry matter production of MSS can be comparable, even under lower fertilizer application rates, with that achieved using grass-based swards, whereas the nutritional quality is generally enhanced because the greater diversity of plant species results in a higher diversity of minerals and phytochemicals [12,13]. In dairy systems, MSS have been shown to maintain milk production compared with rye-grass pastures, whereas urine nitrogen concentration, nitrogen leaching from soils, and the emissions of nitrous oxides are all reduced [14–18]. Because of the greater taxonomic and structural diversity of plants in MSS, below-ground invertebrate and microbiome diversity is also enhanced, and the presence of entomophilous flowering species can increase the above-ground biodiversity, for example, of pollinating insects [19]. Thus, multispecies swards can provide a means by which farmers can reduce their environmental footprint and enhance the farm biodiversity and natural capital, while essentially maintaining the farm's profitability [2].

To reduce or remove the inputs of synthetic fertilizers, farm management systems based on organic and/or regenerative principles advocate for the incorporation of materials that help to maintain soil fertility, organic content, and basal nutrient levels [20]. A more recent aim is to increase soil carbon levels, which both promotes a diverse and functioning ecosystem of soil invertebrates and microorganisms and also helps to reduce the overall carbon footprint of the farm [20,21]. The environmental and financial benefits of incorporating circular economy principles into food production systems are already being discussed and, where possible, additional environmental gains can be obtained if the organic soil amendments are repurposed waste products from another process [22,23]. An obvious example of this circular economy mindset is the traditional application of farm slurry, a waste product from livestock farming repurposed as an organic fertilizer and, as such, allocated an economic value [24]. Farmers are now seeking new soil amendments, ideally repurposed organic by-products, that can provide multiple agronomic and environmental benefits in addition to promoting plant growth by improving the soil health, enhancing the soil microbiome, and providing a means of enhancing the soil carbon.

One potential soil amendment of increasing interest is organic fertilizer based on insect frass. The industrial-scale farming of insects for human food and animal feed is a rapidly growing concern and produces considerable amounts of organic waste as a by-product of the insect rearing process. This frass is dominated by insect feces, itself high in nitrogen, but also contains insect exuviae, exoskeleton fragments, the remnants of the diet material, and, when untreated, a diverse community of microorganisms [25,26]. Frass is considered to have good potential as a sustainable soil amendment and plant fertilizer, and a wide range of plants species, including vegetables, herbs, and grains, have been shown to respond positively to frass application [26,27]. Of additional interest is that frass has been shown to positively affect the soil structure, water retention, and microbial diversity, and may enhance plant resistance to pests and diseases [26,28,29]. The creation and use of insect frass fertilizers (IFF) are often cited as a classic example of circular economy principles, as waste from agriculture or food processing is converted into a new commodity, the insects are used as animal feed, and the waste from this process is converted into a fertilizer that results in the production of more food for humans [27,30,31]. There are even suggestions that farm waste, such as manure, could be directly transformed into an insect-rearing diet, thus allowing farmers to create a high-protein livestock feed and nutrient-rich IFF on-site [32].

Biochar is produced through the pyrolysis of organic biomass at relatively low temperatures (<700 °C) and has been studied extensively for its potential to provide agronomic and environmental benefits [33,34]. The addition of biochar to agricultural soils or plant-growing media has been shown to enhance the performance of multiple plant species, with several mechanisms being proposed for these positive effects, such as increasing the soil water holding retention, decreasing the loss of soil nitrogen and phosphorous, increasing the nutrient availability to plants, reducing the soil acidity, and inducing beneficial changes to the soil microbiota [35,36]. As biochar is repurposed from organic waste, it can be viewed both as means of carbon sequestration in regenerative farming systems and as part of circular bio-economy farming [37].

The general objectives of this study were to obtain data on the responses of two MSS forage herbs, chicory and plantain, to the application of two repurposed soil amendments, insect frass fertilizer and biochar. Both chicory and plantain have long been studied in terms of their value as forage plants, their nutritional properties, their beneficial effects on animal health, and their potential to reduce nitrogen loss via livestock urine (e.g., [38–44]). As MSS are subject to grazing or cropping, it is also important to examine how these soil amendments affect both the initial shoot growth of seedlings and the regrowth of plants after cutting (e.g., [45]). Additionally, as the positive effects of organic fertilizers may only be apparent when the plants are in suboptimal growing conditions, it is valuable to assess the effects of these soil amendments when the plants are maintained in high- and low-nutrient growing media [46].

Specifically, the aims of this study were to use glasshouse trials to: (1) examine the survival and growth of plantain and chicory seedlings after the application of insect frass fertilizer at different rates; (2) investigate the effect of frass fertilizer on seedling growth when applied to growing media with different basal nutrient levels; (3) compare the plant growth obtained with frass fertilizer to that obtained using a standard organic fertilizer; (4) examine the effect of frass fertilizer on the regrowth of chicory and plantain after harvest; and (5) investigate the combined effects of frass fertilizer and biochar on plant growth.

2. Materials and Methods

2.1. General Methods

Trials were conducted in glasshouses at Rosemount Environmental Station, University College Dublin, between February and June 2022. During this period, the glasshouse had a mean temperature of 18.6 °C and relative humidity of 51.2%. No artificial lights were used throughout the trials. Although the suite of experiments was performed over a five-month period, each individual trial only lasted for approximately seven weeks. In each trial, all the plants subjected to the different fertilizer and biochar treatments were grown at the same time, therefore experiencing the same background environmental conditions, meaning that they could be directly compared.

In most trials, we used a potting mix consisting of equal parts by volume of Westland Nutrient Rich Garden Soil, Plagron Coco Bric coir fiber, and vermiculite. The coir fiber and vermiculite contained no nutrients and, although the garden soil was high in humus and 'natural slow-release nutrients', as the garden soil only made up one-third of this growing medium, it was designated as 'low-nutrient' potting mix. In one trial, a 'high-nutrient' potting mix was created by replacing the no-nutrient coir fiber with Westland Multi-Purpose Compost and mixed in a volume ratio of 2:1:1 with the Westland Nutrient Rich Garden Soil and vermiculite. The multi-purpose compost is very high in organic matter (70% peat) and thus produces a richer, more nutrient-dense growing media. As a confirmation of the difference in the nutrient content between the two types of growing media, we observed that the shoot dry weight of both test plants in the high-nutrient mix was over twice that in the low-nutrient growing medium.

HexaFrass™ fertilizer (HF) was obtained from HexaFly, Meath, Ireland (hexafly.com), which is produced by the mass rearing of black soldier flies (*Hermetia illucens* L.), primarily on brewery waste. HF is certified as being organic and described as a slow-release fertilizer which contains approximately 60% organic matter, including some protein and chitin. HF has an NPK of approximately 3.5-2-1, and of the 3% total nitrogen, 2.3% is described as insoluble or slow-release and 0.7% as soluble N and fast-release. An additional analysis by HexaFly indicated that HF contains other important plant nutrients, such as sulfur (6 g/kg), magnesium (5 g/kg), iron (300 mg/kg), and copper (12 mg/kg) (Alvan Hunt personal communication, 19 October 2021). The biochar was manufactured by Argina Fuels, Roscommon, Ireland, and supplied by Biochar Ireland, Lough Derg, Ireland. The biochar was made from hardwood and olive stones using a Kon Tiki cone and then quenched using water. Because the quenching process resulted in a high water content (>50%) of the final product, the biochar was first dried at 105 °C for 3 days and then ground to a fine powder using an electronic grinder before being used. In one trial, a commercial organic fertilizer was used as a positive control. This consisted of Westland Organic Chicken Manure Pellets (chicken manure; CM), which have an NPK of 4.5-3.5-2.5. Biochar powder, HF, and CM were added to the pots prior to seedling introduction and thoroughly mixed into the potting medium.

Non-organic chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) seeds were obtained from Fruit Hill Farm, Bantry, Co Cork, Ireland. The seeds were germinated in shop-bought garden soil in trays prior to being used in the experiments. When the seedlings reached the two-true leaf stage, they were transferred to 7 × 7 cm plastic pots for the experiments, with a single plant in each pot. Each pot was then placed into an individual foil tray (10 cm diameter) to avoid contamination between pots and so that any leached nutrients might be reabsorbed. In all but one trial (see below), the plants were harvested 7 weeks after being transplanted.

In general, the plant growth achieved under the various treatments was assessed in terms of the dry matter accumulation in the shoot. At harvest, the shoots and foliage ('shoot') were cut at the soil surface, and in some trials, the shoot fresh weight (fwt) was obtained. All shoots were placed in paper bags and dried in an oven at 65 °C for 3 days, and the dried shoot weight (dwt) to the nearest mg was obtained using an electronic balance (Bonvoisin Electronic Analytical Balance). In some cases, the shoot dry matter content (%) was then calculated as 100 × (dwt/fwt).

2.2. The Effect of HexaFrass™ Fertilizer on the Plant Performance

To examine how the growth of chicory and plantain responded to increasing amounts of frass fertilizer, HF was applied at the following rates to the standard low-nutrient potting medium: 0, 0.5, 1, 2, 4, 6, 8, and 10 g per pot. There were eight replicates per plant and per application rate ranging between 0 and 6 g per pot and four replicates per plant for the 8 g and 10 g application rates.

To further establish whether HF could achieve similar effects on the shoot growth compared with a standard organically certified fertilizer, a second trial was performed, where chicory and plantain seedlings were grown in the low-nutrient potting mix with the addition of four fertilizer treatments: 0 g HF, 2 g HF, 4 g HF, or 2 g of ground chicken manure (CM). Each fertilizer treatment was replicated 10 times for each plant species.

To examine whether any positive effects of HF on the plant performance were mediated by the basal nutrient levels of the potting media, additional plants were grown in the high-nutrient potting medium (described above) with the addition of 0 g HF or 4 g HF. Each potting mix and fertilizer combination was replicated 10 times for each plant species.

2.3. The Effect of HexaFrass™ on the Regrowth of Chicory and Plantain after Cutting

As chicory and plantain, in the context of this investigation, were considered as forage herbs, it was considered important to examine how HF affected the regrowth of the plants after the shoots had been cut in order to simulate the effects of grazing or cutting for silage. Therefore, additional plants were grown using the low-nutrient potting mix with the addition of 0 g HF or 4 g HF. The shoots of these plants were harvested on two occasions: a first cut was performed 5 weeks after transplantation into the pots, and a second cut performed 7 weeks after transplantation. The effects of HF on the initial growth, regrowth, and total growth were then assessed. There were 10 replicates for each treatment for both plant species.

2.4. The Effect of HexaFrass™ Combined with Biochar on the Growth of Chicory and Plantain

To examine whether the application of biochar alone or in combination with HF affected the growth of chicory and plantain, plants were grown using the low-nutrient potting media. A factorial experimental design was used, in which powdered biochar was added at rates of either 0 g or 2 g per pot, whereas HF was applied at 0, 1, 2, or 4 g per pot. Each of these eight combinations was replicated 10 times for each plant species.

2.5. Data Analysis

All the data were organized using Microsoft Excel, and the statistical analysis performed using Genstat software (v 21, VSNI, Hemel Hempstead, UK). For each trial, the data for chicory and plantain were analyzed separately. For most trials, the data were subjected to one-way or two-way ANOVA, and pairwise comparisons of the treatments were performed using Fisher's least significant difference (LSD) at $p < 0.05$. For all the ANOVA tests, the conditions of equal variance and a normal distribution of residuals were verified through the visual inspection of the distribution of residuals, and no data transformations were used [47].

In the regrowth trials, the shoot growth in the two HF vs. no-HF treatments was initially compared using unpaired t-tests, assuming unequal variances. A second analysis comparing the effects of two factors, HF and the cutting regime (one cut vs. two cuts), on the total yield was performed using two-way ANOVA.

In the examination of the effect of the HF application rate on the shoot dry weight (dwt; mg), polynomial regression curves were fitted for each response variable using a quadratic model of the form:

$$\text{Shoot dwt} = a + b(\text{HF}) + c(\text{HF}^2).$$

where a, b, and c are constants and HF is the application rate in g. For these polynomial relationships, HFMax, the HF application rate (per pot) that can produce the maximum mean shoot dry weight, can be estimated using the formula $-b/2c$.

To examine how the HF application rate affected the shoot dry matter content (DM, %), exponential asymptotic curves were fitted in the form:

$$\text{Shoot DM} = d - e.f^{\text{HF}}$$

where d, e, and f are constants and HF is the application rate per pot in g, a being the asymptote of the curve and c being a positive constant < 1.

For one trial, whereby we wanted to show how the variation differed between treatments, the coefficient of variation (CV%) was calculated as = 100 × standard deviation/mean.

3. Results

3.1. The Effect of HexaFrass™ on the Performance of Chicory and Plantain

Plant survival remained at 100% up to 4 g HF per pot for chicory and 6 g HF per pot for plantain. After these application rates, the plant survival decreased steadily with additional HF, and only one of the four plants (25%) survived when 10 g HF was applied to both the chicory and plantain (Figure 1a,b).

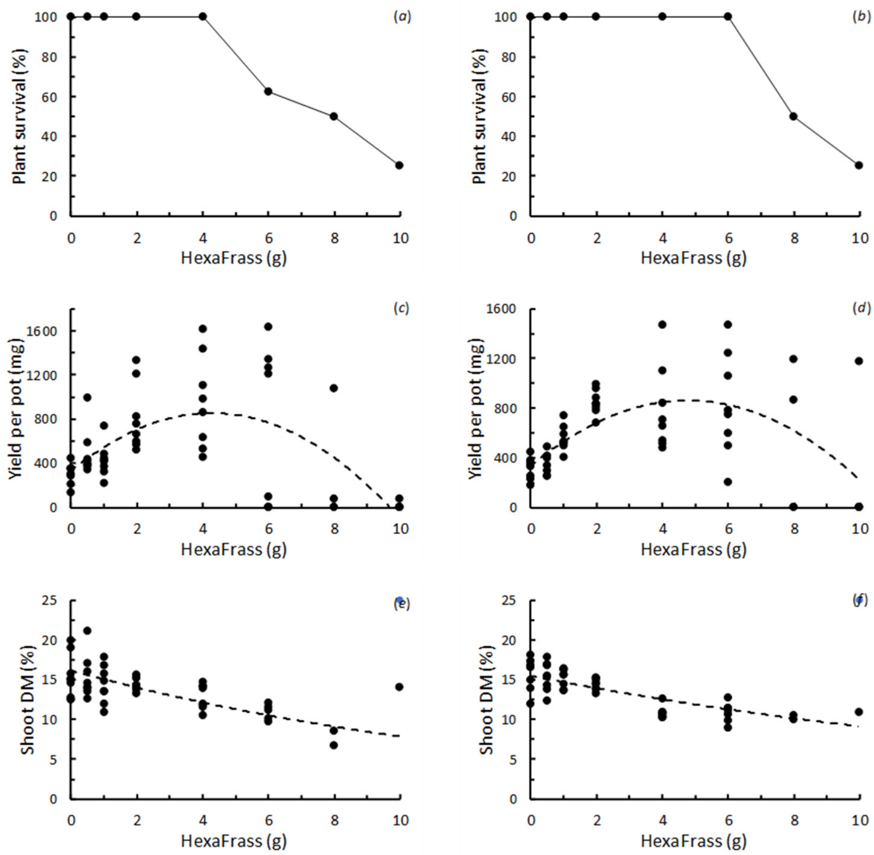

Figure 1. The effect of adding insect frass fertilizer (HexaFrass™; g per pot) on the survival to harvest, shoot yield per pot (mg), and percentage shoot dry weight of chicory (**a**,**c**,**e**) and ribwort plantain (**b**,**d**,**f**) grown under glasshouse conditions.

In terms of the shoot yield per pot, there was an initial increase in the shoot dwt with the addition of HF up to approximately 4 g per pot. As the plant survival decreased when 8 g and 10 g of HF were added, the average yields then also began to decrease. Additionally, some plants subjected to the higher HF application rates were sickly, and this resulted in low shoot weights (Figure 1c,d). The relationship between the yield (dwt; mg) per pot and applied HF could, therefore, be described by polynomial equations (Figure 1c,d) in the forms:

$$\text{Chicory: Yield} = -28.99\text{HF}^2 + 246.81\text{HF} + 329.47$$

$$\text{Plantain: Yield} = -23.32\text{HF}^2 + 221.89\text{HF} + 333.73$$

These equations provided the estimates of the optimum amount of HF that could maximize the shoot yield as 4.36 g per pot for chicory and 4.8 g per pot for plantain, ≈1% of the volume of the growing media.

Although the mean shoot yields per pot increased as up to 4 g per pot of HF was applied, the variability in the yield also increased. Thus, for chicory, the coefficient of variation (CV%) of the control plants was 31% compared with 44% when 4 g HF was applied. Similarly, for plantain, the CV% of the control plants was 28% compared with 44% when 4 g HF was applied per pot.

The shoot dry matter content (DM%) decreased with the increasing quantity of applied HF, a relationship which could be modelled using asymptotic exponential curves (Figure 1e,f). At 10 g HF, there was a slight increase in the (DM%) for both chicory and plantain, as these plants showed signs of ill health and likely had some dead foliage.

3.2. A Comparison of HexaFrass™ and a Standard Organic Fertilizer on the Shoot Growth of Chicory and Plantain

The addition of 2 g HF, 4 g HF, and 2 g CM all significantly increased the shoot dry weight of both chicory ($F_{3,35} = 18.3$; $p < 0.001$) and plantain ($F_{3,32} = 27.2$; $p < 0.001$; Figure 2). In both plant species, the effects of adding 2 g or 4 g of HF were broadly similar to those of adding 2 g of CM. Nevertheless, in chicory, the shoot growth was significantly increased (≈29%) by the 4 g HF treatment compared with 2 g CM, whereas for plantain, it was the 2 g HF treatment that produced a significantly greater (≈25%) shoot growth than the CM treatment (Figure 2). In both plants, adding 4 g HF did not cause a significant increase in the shoot dwt compared with adding 2 g HF (Figure 2; see also Figure 1c,d).

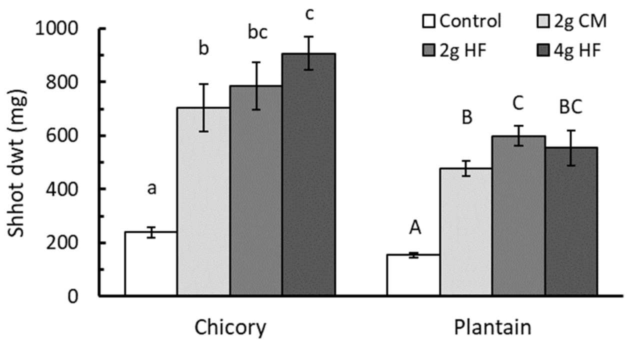

Figure 2. The effect of adding HexaFrass™ (HF; 2 g or 4 g per pot) and a standard organic fertilizer (chicken manure, CM; 2 g per pot) on the shoot growth of chicory and plantain grown under glasshouse conditions (mean ± SE). Only data for plants that survived until harvest are included. Letter codes separate treatments for each plant using LSDs at $p < 0.005$.

3.3. Effect of HexaFrass™ on the Shoot Growth in High- and Low-Nutrient Potting Mix

As expected, when no HF was applied, the shoot growth was significantly higher for both chicory (2-fold) and plantain (3-fold) in the high-nutrient growing medium compared with the low-nutrient growing medium (Figure 3). Additionally, there were significant statistical interactions between the effects of the HF application and the basal nutrient content of the potting mix for both chicory ($F_{1,34} = 102.7$; $p < 0.001$) and plantain ($F_{1,31} = 21.3$; $p < 0.001$; Figure 3). The positive effects on the shoot growth observed after adding 4 g HF per pot to the low-nutrient potting mix were not seen in the case of the high-nutrient potting mix. In plantain, this resulted in the separation of the low-nutrient soil with no HF treatment from the remaining three treatments. For chicory, the addition of the HF to the high-nutrient potting mix caused a reduction in the average shoot growth, with some plants remaining very stunted (Figure 3).

The data presented in Figure 3 summarize the shoot dry weight data only for plants that survived until the final harvest. However, four of the ten chicory seedlings and three of the plantain seedlings did not survive in the high-nutrient soil + 4 g HF combination, whereas all plants survived in the high-nutrient soil with no HF added. Thus, if the yield per pot is considered, the higher plant mortality observed in the HF-treated plants caused an additional reduction in the average yields. Thus, for chicory, in the high-nutrient soil, the yield per pot was 793 ± 120 SD mg when no HF was added, compared with 225 ± 261 SD mg with 4 g HF. Similarly, for plantain, in the high-nutrient soil, the yield per pot was 640 ± 360 SD mg when no HF was added, compared with 102 ± 337 SD mg when 4 g HF was applied.

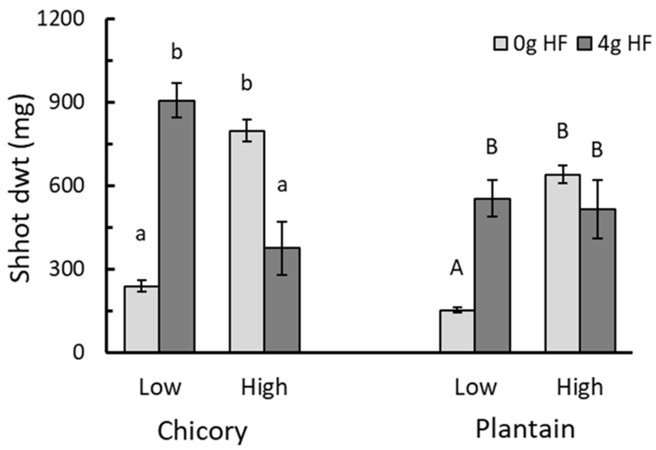

Figure 3. The influences of soil nutrients (Low vs. High) and HexaFrass™ (0 g vs. 4 g per pot) on the shoot dry weight (mg) of chicory and plantain grown under glasshouse conditions (mean ± SE). Only data for plants that survived until harvest are included. Letter codes separate treatments for each plant using LSDs at $p < 0.05$.

3.4. Effect of HexaFrass™ on the Shoot Growth of Chicory and Plantain after Cutting

The addition of 4 g HF increased the shoot dwt of chicory after five weeks (1st Cut) and two weeks of regrowth (2nd Cut), which resulted in a total increase of 193% (Figure 4). For plantain, although there was no significant effect of adding 4 g HF on the five-week harvest (1st Cut), HF did significantly increase the regrowth (2nd Cut), resulting in a total increase of 90% (Figure 4).

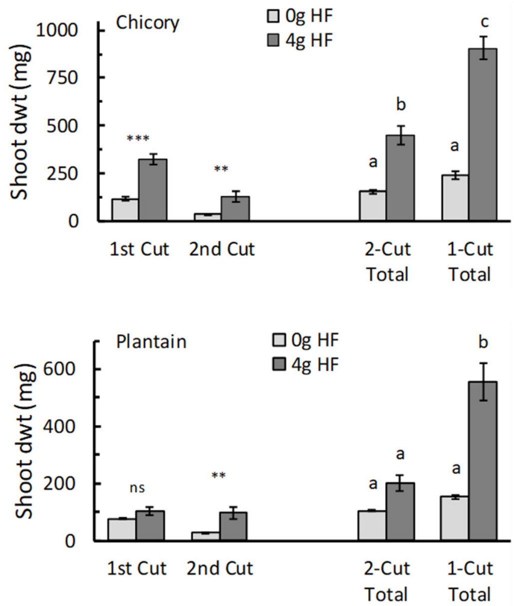

Figure 4. The influence of HexaFrass™ (4 g per pot) on the shoot growth (mean ± SE) of chicory and plantain at five weeks (1st Cut) and after two weeks of regrowth (2nd Cut). The total shoot growth of the cut plants (2-Cut Total) and plants that were harvested only once after seven weeks (1-Cut Total) are also presented. Difference between 0 g and 4 g HexaFrass™ treatments tested by unpaired *t*-tests; ns—no significant difference, ** $p < 0.01$, *** $p < 0.001$. Total harvests compared using ANOVA with HF (0 g vs. 4 g) and the cutting regime (1-cut vs. 2-cut) as factors. Letter codes separate treatments for each plant using LSDs at $p < 0.005$.

When considering only the total harvests, there were significant statistical interactions between the HF treatment and cutting regime for both chicory ($F_{1,34} = 19.6$; $p < 0.001$) and plantain ($F_{1,34} = 19.2$; $p < 0.001$). Overall, the largest shoot weights for both plant species were obtained from plants which were treated with HF and harvested only once (Figure 4). For the control plants with no HF applied, there was little difference in the total yield between plants that were harvested once or twice (Figure 4). However, the application of 4 g HF to the twice-cut plants produced shoot weights that were similar in weight in the case of plantain and greater in weight in the case of chicory than the control plants subjected to only a single harvest (Figure 4).

3.5. Effect of HexaFrass™ and Biochar on the Shoot Growth of Chicory and Plantain

For both chicory and plantain, adding increasing amounts of HF caused a significant increase in the average shoot dry weight (Chicory $F_{3,63} = 37.0$, $p < 0.001$; Plantain $F_{3,63} = 34.8$, $p < 0.001$; Figure 5). For both plant species, there was no significant effect on the shoot growth caused by adding 2 g powdered biochar when no additional HF was applied (i.e., the 0 g HF treatments; Figure 5). However, for chicory, taking all the HF treatments into consideration, the addition of 2 g biochar per pot caused a significant increase (≈25%) in the shoot dwt ($F_{1,63} = 7.4$, $p = 0.008$), although a significant pairwise separation of the biochar treatments within the spectrum of HF application rates was observed only when 4 g HF was applied (Figure 5).

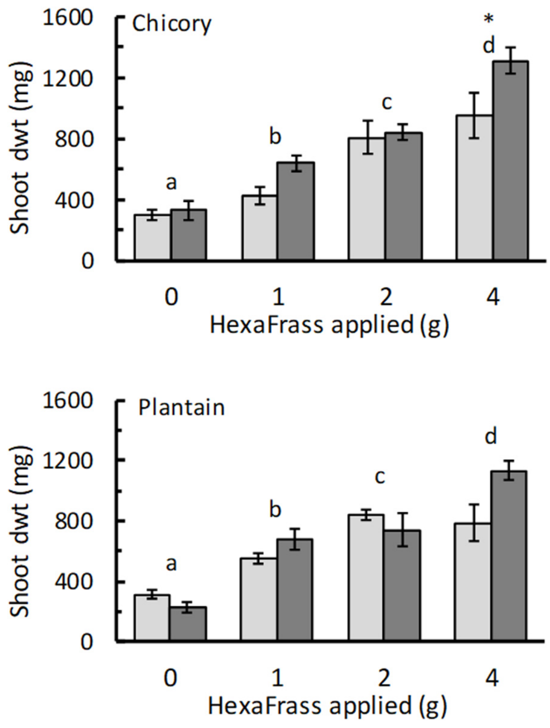

Figure 5. The influence of applying HexaFrass™ (HF) and biochar (light columns 0 g; dark columns 2 g) on the shoot growth (mean ± SE) of chicory and plantain. Pairwise comparison of treatments performed using LSDs at $p < 0.05$. Letter codes separate HF treatments for each plant. Significant differences between 0 g and 2 g biochar treatments for each HF application rate are indicated by *.

For plantain, there was no simple overall effect of adding biochar ($F_{1,63} = 2.0$, $p = 0.166$), and there were no significant pairwise differences observed within any one HF application rate (Figure 5). There was also some inconsistency in the effects of adding biochar to plantain, in that biochar caused a slight reduction in growth using the 0 g and 2 g HF treatments, and a slight increase in growth using the 1 g and 4 g HF treatments (HF × Biochar interaction, $F_{3,63} = 4.5$, $p = 0.007$; Figure 5).

4. Discussion

Considering the results of the multiple trials performed in this investigation, the general conclusion was that HF, an insect-frass-based fertilizer, significantly enhanced the shoot growth of two forage plants, chicory and plantain. This result corroborates the findings of several other recent studies, where the application of insect frass fertilizer positively affected the growth of herbs, vegetables, and cereals [25,27,48–52]. The positive effect of HF on the shoot growth of chicory and plantain was broadly comparable to that achieved after the application of a similar amount of recognized organic soil amendment, commercially produced chicken manure pellets. This finding was similar to previous results of studies where the shoot growth of basil (*Ocimum basilicum* L.), lettuce (*Lactuca sativa* L.), and parsley (*Petroselinum crispum* (Mill.) Fuss) was similarly affected by the application of HF and chicken manure [27].

As a result of both higher plant mortality and smaller shoot growth at high application rates, the yield per pot of plantain and chicory showed non-linear polynomial relationships with the amount of HF applied, with optimal application rates being in the range of approximately 4–5 g per pot for both species. Non-linear relationships between the shoot dry weight and HF were also found by Borkent & Hodge [27], and other studies have also reported stunting effects on the plant growth when black soldier fly frass was applied at high doses [50,51]. Additionally, in the current study, the application of HF to growing media with high basal organic/nutrient contents also had a negative effect on the plant growth. Taken together, these results indicate that these forage species may not respond well to the excessive application of IFF, especially in rich soil, in soil with a high organic content, or when the plants experience no significant nutrient deficits. Other studies have shown positive relationships between N supply and shoot growth of both chicory (e.g., [53]) and plantain (e.g., [54]). As the N content of HF is only ≈4%, it seems unlikely that the negative impacts of the high HF application rates were due to excessive N. It is possible that the plants responded to other chemicals present in the HF or the creation of a growing media that is too rich in organic matter. These suggestions are, however, speculative, and more research is needed to clarify how these plants respond to high doses of insect frass and to assess the survival/growth responses compared with those seen when N and other nutrients are applied directly in mineral form.

The incorporation of HF into the plant growing medium caused an approximately 3-fold increase in the re-growth of both chicory and plantain after cutting compared with that obtained in the control plants. This effect has important ramifications for grazing systems, as it suggests that HF could shorten the interval between consecutive cuttings or grazing events [55]. There was little difference in the total harvested shoot weights of both plants after a single harvest or two cuttings when HF was not applied. The application of HF resulted in a doubling of the total harvested shoot weight after two cuts for plantain and a tripling for chicory, but the largest HF-induced gains were still seen when only a single harvest was performed. This latter result suggests that farmers could benefit from allowing these plants to undergo a longer regrowth period, as this would considerably increase total dry matter yields. However, this strategy would only be relevant if HF or another organic supplement were to be applied, reaffirming that the optimization of MSS management requires consideration of both the plant species present and of the fertilizer regime [55,56].

The biochar, on its own or in combination with HF, did not have such dramatic effects on the shoot growth of either chicory or plantain compared with those obtained with HF. Several studies have found that the application of biochar to cereals, for example, did not produce increases in the above-ground biomass or yield [18,57,58]. Conversely, other trials have shown increases in crop biomass when biochar was applied in conjunction with organic materials, such as farmyard manure [59], compost [60], manure [61] or synthetic fertilizers [62]. In our study, there were indications to suggest that, when biochar was added to growing media that also contained 4 g HF (close to the optimal HF application rate identified in the dose–response trials), it resulted in an increase in the shoot growth in addition to that caused by the HF alone. At the very least, the results suggest that farmers aiming to increase the carbon content of their soils by applying biochar and, thus, reduce their farms' overall carbon footprints, could do so without negatively impacting the growth of these forage species.

We concede that there are several limitations to our study with respect to the extrapolation of our findings to full farm conditions. For example, the trials were performed in relatively small containers under benevolent glasshouse conditions, where biotic and abiotic stresses on the plants were minimized, and the time frames were limited to a period of around 60 days between the sowing of seeds and harvesting of shoots. We also recognize that plant responses were measured only in terms of the shoot dry weight and dry matter content, and the evaluation of these species as forage for livestock would also require the assessment of changes in foliage chemistry and nutritional value. Nevertheless, organic amendments and biostimulants, such as those based on seaweed and humic substances, although much vaunted, do not always produce positive effects on plant growth (e.g., [46,63]). Therefore, 'pot trials' such as those used in this investigation can offer a rapid and cost-effective screening process for new plant-growth-promoting products based on insect frass. and aid in the identification of both potential benefits and possible issues such as the reduction in plant performance at high application rates (e.g., [64,65]).

The evaluation of IFF is a fairly recent endeavor, and much future research is needed to gain a more holistic picture of how MSS might react to IFF. Future work would benefit from screening for consistencies in the effects among different brands of IFF, as frass produced by different insect species or produced using different rearing diets can result in variable organic and nutrient contents [66,67]. Similarly, although most IFFs are applied in dry form, there may be additional or alternative advantages to applying frass extracts as foliar drenches, as this can affect micronutrient absorption and potency of plant defense activators [68–70].

5. Conclusions

Overall, the results reported here indicate that IFF can increase the shoot dry matter of two forage species, chicory and plantain, and also increase shoot regrowth after grazing or harvesting for silage. The application of IFF has also been shown to increase the growth of other pasture species, such as rye grass, and increase the biomass and nutrient content of field-harvested forage (e.g., [65,71,72]). Thus, although the uptake of IFF by farmers may currently be limited by the rate of production, as insect farms become more common and these products become more available, IFF appears to offer good potential as an organically acceptable soil amendment in low-input MSS grazing systems.

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