Volatile organic compounds emitted by invasive and native plant species under invasion scenarios and their potential ecological roles

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Evans Effah

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Summary

Climate change, human migration, and global trade favour the spread of plant species beyond their natural ranges. Many of these plants become invasive, posing a risk to the persistence and survival of native species and the ecosystems they invade. In New Zealand, the European woody shrub *Calluna vulgaris* (heather) is the most widespread invasive weed on the Central Plateau of North Island. Like most exotic invasive plants, the chemical behaviour (i.e. chemical production and chemical mediated interactions) of heather in its invaded habitat is poorly understood. Moreover, despite the struggles of native plants to endure the stress induced by exotic weeds, no study has documented the chemical behaviour of native plant species in plant invasion scenarios. Volatile organic compounds (VOCs) are secondary plant metabolites that play a vital role in plant communication with other organisms and are highly responsive to biotic and abiotic stress. Therefore, measuring VOC emissions during plant invasion could provide valuable information about plant responses to the changing environment and their potential impacts on other community members. This thesis aimed to investigate VOCs emitted by the invasive weed heather and a New Zealand native plant *Leptospermum scoparium* (mānuka) under field conditions, while determining the environmental factors regulating their emissions and exploring their potential ecological impacts under lab and field conditions. Results from the field trials on the Central North Plateau showed variations in the volatile profiles of heather and mānuka growing at different sites, with both plants emitting lower amounts of VOCs at sites where other exotic

invasive plants were present. This reduction in VOC emissions was mostly due to indirect changes in environmental factors, like soil properties, which were driven by the invasive weeds heather and *Cytisus scoparius* (Scotch broom; henceforth broom). This thesis also documents the chemical responses of heather to two major stresses encountered in New Zealand; 1) elevated solar ultraviolet radiation (UV) and 2) damage caused by its introduced specialist herbivore and biocontrol agent *Lochmaea suturalis* (heather beetle). Results from these trials demonstrate that high UV radiation reduced the volatile emissions of some compounds (mainly terpenoids) and that the impacts of herbivory by heather beetle on VOC emission depended on the developmental stage of the herbivore, plant phenology, and season. The ability of VOCs emitted from heather and broom to affect the germination and growth of mānuka was also tested in the lab, while the impact on arthropod communities were investigated at sites on the Central North Plateau. The results suggest that VOCs produced by invasive plants may have phytotoxic effects toward mānuka and may alter arthropod community structure. This thesis highlights the complexity of plant chemical communication under invasion scenarios and invites further exploration of the interactions between exotic invasive plants and native species to broaden our understanding of invasion ecology to support weed management, biocontrol, and conservation efforts.

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Declaration

This thesis is based on publications, and the formatting style of each chapter follows the guidelines for the journal to which it will be submitted or in which it has been published or accepted for publication. Hence, there are inconsistencies in writing style, referencing format and some repetition of methods between the chapters presented in this thesis. Contributions of authors are specified in each chapter when necessary.

Chapter 1

General introduction

Native tussock grassland at sites where heather is absent (Paradise Valley, Central North Plateau).

Photo credit: ***Paul G. Peterson*** *(Manaaki Whenua) and* ***Paul D. Barrett*** *(Massey University).*

1.1 What are plant volatile organic compounds, and why is it important to study them under invasion scenarios?

Plants convert inorganic carbon to organic molecules, and a significant amount of the carbon that is fixed is re-released into the atmosphere as volatile organic compounds (VOCs) (Holopainen, 2004). Volatile organic compounds, like other plant secondary metabolites, may not be involved directly in plant growth and reproduction but are vital to plant communication and defence against biotic and abiotic stress (Pichersky & Gershenzon, 2002; Dudareva et al., 2006).

Evidence over the last four decades suggests that plant volatiles are crucial in plant communication, mediating several above and belowground interactions between plants and surrounding organisms (Pichersky & Gershenzon, 2002; Holopainen, 2004; Dudareva et al., 2006). Plant volatiles (especially those in flowers and fruits) are well known to attract pollinators and seed dispersers, contributing to reproduction in angiosperms, but VOCs are also emitted from vegetative plant organs (leaves, stems and roots) (Dicke & Baldwin, 2010; Rowan, 2011; Rashid & Chung, 2017).

The composition of volatile blends is species-specific, as they depend on the biosynthetic ability of a plant to produce them. However, plant volatile emissions vary between cultivars, gender, and plant organs (Flamini et al., 2007; Vivaldo et al., 2017; Moreira & Abdala-Roberts, 2019), and in response to biotic and abiotic variables such as herbivory or pathogen infection, soil composition, temperature, drought and UV-radiation. This makes plant volatile emissions an

excellent source of information about a plant's health and how it is responding physiologically to neighbouring plants and other organisms (McCormick, 2016).

VOCs have been studied extensively in relation to plant defence, and the reports show that damaged plants reduce herbivore loads by emitting herbivore-induced volatile blends that repel herbivores and attract natural enemies (Dicke & Baldwin, 2010; McCormick et al., 2014; Turlings & Erb, 2018). Furthermore, undamaged neighbouring plants can detect these herbivore-induced volatiles and enhance their defence state in a phenomenon called priming (Heil & Kost, 2006; Ton et al., 2007), indicating that plants do not only emit volatiles but are able to detect and respond to them. It has also been hypothesised that plants use volatile cues from their neighbours to monitor and predict potential competitors and prepare physiologically for future competition (Ninkovic et al., 2013, 2016).

Some studies, e.g., Wang et al. (2010) and Araniti et al. (2017) also show that VOCs can have allelopathic properties, inhibiting the growth and germination of neighbouring plants. VOCs could therefore play an important role in plant competition, either directly through allelopathy or indirectly by modifying plant interactions with other organisms (Kegge and Pierik, 2010). The roles of VOCs in plant competition remain largely unexplored, but with the increasing spread of invasive species, it is of paramount importance that we attempt to understand the complex chemical interactions between invaders, their new environments, and native species.

Climate change and the spread of invasive species, including plants, constitute two significant ways by which humans have modified natural ecosystems. The impacts of climate change, including warming temperatures, changes in UV radiation and elevated CO_2 concentrations, favour the colonisation and spread of invasive alien species that are highly adapted to disturbance and tolerate a wide range of environmental conditions (Bradley et al., 2010; Burgiel & Muir, 2010). Thus, invasive species and their impacts are predicted to increase globally in coming years.

Most introduced plants become invasive in new environments partly because they are fast growers, produce numerous seeds, and display high phenotypic plasticity (Funk, 2008; Van Kleunen et al., 2010). Introduced plants usually escape their specialist herbivores and other natural enemies in the new habitat (Vila et al., 2005; Liu & Stiling, 2006), and can, therefore, allocate more resources to growth and reproduction instead of defence.

Highly invasive exotic plant species can displace native plants resulting in biodiversity loss (Gaertner et al., 2009; Vilà et al., 2011). Native plants are often displaced through direct competition for above and belowground resources (Corbin & D'Antonio, 2010; Bottollier-Curtet et al., 2013), competition or removal of pollinators or other essential mutualists (Muñoz & Cavieres, 2008), and modification of soil properties and microbiota (Lorenzo et al., 2010). Recent studies also show that invasive plants can release phytotoxic compounds into the environment directly inhibiting the germination and growth of native plants

(Callaway & Ridenour, 2004; Murrell et al., 2011), suggesting that chemical warfare is also part of their success strategy.

The functional roles of VOCs in competition between invasive and native plants have been poorly explored and are mostly limited to reports on phytotoxic effects of invasive plants' VOCs on seed germination and growth of native plants (Barney et al., 2009; Inderjit et al., 2011; Araniti et al., 2017). Unfortunately, it remains unclear how new environmental conditions impact the VOC emissions of invasive species, whether native plant species' VOC emissions change in response to the invasion, and what impact these changes have on other community members.

This PhD thesis aims to answer these questions by exploring the volatile organic compounds emitted by invasive and native plant species under different invasion scenarios, and their potential ecological roles on the Central Plateau of North Island, New Zealand.

1.2 Study system

The North Island Volcanic Plateau is a vast area at high altitude in the central part of the North Island, New Zealand. This region is well known for its imposing landscape and three prominent volcanoes; Tongariro (1968 m a.s.l), Ngauruhoe (2291 m a.s.l) and Ruapehu (2739 m a.s.l) (Chapman & Bannister, 1990), which are protected within Tongariro National Park. The National Park was New Zealand's first and due to its scientific, natural and cultural value, was listed as a UNESCO dual heritage site in 1993. The dominant native vegetation

present on the plateau includes *Chionochloa rubra* (red tussock), *Leptospermum scoparium* (mānuka), *Dracophyllum subulatum* (monoao; henceforth *Dracophyllum*), *Poa colensoi* (blue tussock) and some other endemic low growing subalpine species (Rogers, 1994; Blayney, 2012).

However, the intrinsic and aesthetic value of this ecosystem is being threatened by invasion of exotic plants, including heather *Calluna vulgaris* (heather) and *Cytisus scoparius* (broom). Both heather (Ericaceae) and broom (Fabaceae) were introduced into New Zealand from Europe. Heather was deliberately planted in the Tongariro National Park in 1912 to convert the seral tussock grassland into a habitat for grouse hunting (Bagnall, 1982; Chapman & Bannister, 1990), while broom invaded the area in the 1960s but not yet widespread (Buddenhagen, 2000). Since their introduction, heather and broom have proven to be detrimental, modifying soil properties and displacing native flora and fauna (Keesing, 1995; Rogers, 1996; Rogers & Leathwick, 1996).

Despite the risks associated with their invasion, knowledge of the chemical behaviour (i.e. production of chemicals and chemical-mediated interactions) of exotic invasive plants, in general, remains limited, and no study has investigated the factors regulating the volatile organic compounds released by exotic weeds in nature. This information is particularly important, considering that volatile compounds released by weeds into a naïve environment could directly inhibit the growth of native plants and modify the communication between native vegetation and their associated arthropods or microbes, contributing to the success of the invaders.

1.3 Aims

Given that plant volatiles are crucial in plants communication (Pichersky & Gershenzon, 2002; Heil & Karban, 2010), that they are highly responsive to biotic and abiotic changes (Holopainen & Gershenzon, 2010; McCormick, 2016), and that the composition and relative amounts of compounds in a volatile blend may be dependent on the species composition of neighbouring vegetation (Kigathi et al., 2019), it is important to measure the volatile emission during plant invasion. Changes in these emissions may influence the behaviour of resident organisms (microbes, plants and animals) and their ecological interactions. Therefore, this study aims to:

1. Characterise the volatile emissions of heather and identify environmental factors regulating them.
2. Investigate the chemical response of invasive plants (heather) to abiotic (UV radiation) and biotic (herbivory) stressors in the invaded range.
3. Explore the volatile emissions of native plants (mānuka) under invasion scenarios.
4. Investigate the response of the arthropod community to plant invasion and explore other potential ecological impacts of volatiles emissions during plant invasion.

1.4 Thesis structure

This thesis consists of seven Chapters. Chapter 1 is the general introduction, Chapter 2 is a literature review of how plant volatiles can influence the outcome

of plant competition. Chapters 3, 4, 5 and 6 are research-based papers targeting the specific aims of the thesis. Chapters 2, 3, 4, 5 and 6 have been published in peer-reviewed journals. Chapter 7 is a general discussion. Appendix 5 is a small experiment conducted to support discussion material in the thesis. A summary for each chapter is presented below.

Chapter 1 provides vital background information on the concepts presented in the thesis. Topics introduced in this chapter include exotic invasive plants, their impact on invaded habitats and how they produce volatile organic compounds (VOCs), and their ecological functions. This chapter also summarises the evidence on volatile-mediated interactions in plant invasions. Finally, details are provided about the sites where the field experiments were conducted.

Chapter 2 reviews the literature on the role of VOCs in plant competition. It explores the current state of knowledge on plant competition and VOCs and identifies gaps in the field. This chapter also provides evidence on how VOCs could help shape the competitive outcome between plants and provides recommendations for research needed to enhance our knowledge in this field.

Chapter 3 investigates the volatile emissions of *Calluna vulgaris* (heather) and the environmental factors that regulate these emissions on the Central North Plateau of New Zealand. Volatiles produced by heather at four different sites were measured in the summer of 2017 – 2018. Each site was distinct and characterised by the combination of heather either with either conspecifics or heterospecifics. Environmental factors including soil water content and

nutrients, air temperature, and herbivore damage on heather, were also measured at each site and their effect on volatile emissions determined. The results show that volatile emissions by heather are largely affected by the level of soil nutrients between the sites, with lower emissions where heather co-occurs with another invasive plant (broom).

Chapter 4 investigates the effect of UV radiation and herbivore damage by the specialist *Lochmaea suturalis* (heather beetle) on the volatile emissions of heather. The UV trial was done by exposing potted heather plants to different UV levels in a tunnel house, while the herbivory trial was conducted at sites where heather beetles were either present or absent. The results of the UV trial suggest that heather emits less VOCs under higher UV levels and results from the herbivory trial show variation in volatile emissions between the sites where the beetle was present and absent.

Chapter 5 characterises for the first time the volatile organic compound emissions from the New Zealand native plant mānuka (*Leptospermum scoparium*), in an invasion scenario. Volatile emissions from mānuka, either in a monospecific stand or where it co-occurs with one of the three heterospecifics (heather, broom or *Dracophyllum*), were measured during summer 2017 and winter 2018. Biotic (herbivory) and abiotic factors (temperature, soil water content and nutrients) were also measured at each site, and their effect on the emitted compounds investigated. Site-specific and seasonal variation in volatile emissions were detected, with lower emissions at sites invaded by heather or broom.

Chapter 6 examines the arthropod community composition at sites where exotic invasive weeds (heather and broom) were either present or absent. Arthropods were collected in summer and autumn using different trapping techniques. Arthropod type and abundance were highly dependent on the species composition of neighbouring plants.

Chapter 7 is a general discussion, and integrates results from all experiments. This chapter also explains the ecological implications of the findings and provides recommendations for future studies.

Appendix 5 is a small study investigating the phytotoxic potential of volatiles released by exotic invasive species on the seed germination and growth of a native plant (mānuka). The trial was conducted in a lab-based growth cage by exposing mānuka seeds to one of the four treatments; clean air, or volatiles produced by mānuka, heather and broom. The preliminary results show a potential adverse effect of the volatiles emitted by the exotic weeds on seed germination and biomass of mānuka. This study was not completed due to COVID-19 lockdowns.

1.5 Ethical issues

This study has no human and animal ethical issues.

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Chapter 2

Potential roles of volatile organic compounds in plant competition

This chapter identifies a gap in our knowledge of competition in plant communities and presents evidence from the literature to illustrate the possible way by which volatile organic compounds could shape the outcome of plant competition. It also suggests multiple avenues for future research in this field. The chapter is presented in the style of the journal Perspectives in Plant Ecology, Evolution and Systematics with minor structural modifications. The chapter was published by the journal *Perspectives in Plant Ecology, Evolution and Systematics* as:

Effah, E., Holopainen, J.K. and McCormick, A.C., 2019. Potential roles of volatile organic compounds in plant competition. *Perspectives in Plant Ecology, Evolution and Systematics 38,* 58 – 63.

Abstract

Volatile organic compounds (VOCs) are a major currency in plant communication where they mediate above- and below-ground interactions between plants and the surrounding organisms (i.e., other plants, microorganisms, pollinators, seed dispersers, herbivores, and their natural enemies). Considering the multiple interactions mediated by VOCs and their impact on a plant's reproductive success and survival, they can be a crucial weapon in plant-plant competition. However, this particular role of VOCs is underrepresented in the literature. Mechanisms by which volatiles can mediate plant competition can be direct or indirect. Direct mechanisms include establishing a neighbour's identity and status to select adequate responses and affecting competitor's seed germination or growth through VOC-mediated allelopathy. Indirect mechanisms can affect the plant's competitive ability by modifying interactions with other trophic levels, for instance, through associational resistance or chemical camouflage. These mechanisms are not mutually exclusive and can be seen as part of a continuum. In this review, we present evidence from the literature to illustrate these roles and indicate how they could influence competition in plant communities. We propose new research avenues to test if and how these mechanisms affect competitive outcomes and suggest that, in addition to morphological traits, future competition studies should also incorporate data on plant volatile emissions and measure their effects on the surrounding plants and other trophic levels. This information would allow us to understand competition from a broader perspective by acknowledging the

existence of multiple (possibly coexisting) competition strategies and the role of other trophic levels in shaping competitive outcomes.

Keywords: Allelopathy, Associational resistance, Competition, Kin recognition, Priming, VOCs

2.1 Introduction

In an ecosystem, plants interact with other organisms, creating a complex network which can influence ecological stability (Mougi and Kondoh, 2012). Plant-plant interactions occur between conspecifics and heterospecifics, and have positive (facilitation), neutral, or negative (competition) outcomes (Kuebbing and Nuñez, 2015). Competition is defined as an adverse effect, in which one organism consumes or controls access to a resource that is limited in availability (Keddy, 1989), and it is one of the most studied phenomena in plant ecology, as it influences the abundance and distribution of species, making it a prevalent force in the structuring of plant communities (Alexandrou et al., 2011).

To get an idea of the current state of knowledge on competitive interactions between plants, we searched for primary literature on Scopus by using the keywords ‘Plant’ AND ‘Competition’ in the article title. The search was limited to studies published over the last decade (2008 to 23rd October 2018) and language was limited to English. Using these search criteria, we obtained 219 studies in which the authors investigated intra- or interspecific competition between plants. The data reveal that most competition studies (45%) focus on

growth, biomass and morphological traits (such as leaf length, specific leaf area, and root length), while other aspects represent only smaller fractions of the available research (Fig. 1). Relying on growth, biomass and morphological changes alone can provide only a limited understanding of how complex ecological interactions shape plant competition. For instance, plant secondary metabolites are involved in a wide array of plant-biotic interactions ranging from defence against herbivores to pollinator attraction (Pichersky and Gershenzon, 2002) and could thus provide much needed information on the broader ecological context of plant competition. However, only a minority of studies in the last decade (3.7%) have investigated secondary metabolites in plant-plant competition, and of this proportion, only a small fraction has explored volatile organic compounds (VOCs) and their ecological roles (Fig. 1).

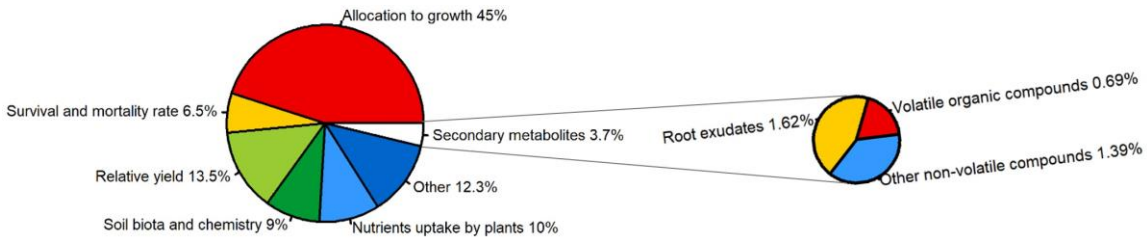

Figure 1. Common traits measured in plant-competition studies based on 219 studies within the last ten years resulting from a Scopus search conducted in October 2018 using the terms 'Plant' AND 'Competition' in the article title.

VOCs are emitted by all plant taxa both constitutively, and in response to biotic and abiotic stress (Holopainen, 2004), and are regarded as signals (or cues) mediating communication between plants and other organisms, as well as "bioactive" compounds that elicit responses in the receiver. VOCs play a crucial

role in plant-biotic interactions, such as host selection by insects (Bruce et al., 2005), acting as feeding, habitat, and oviposition cues for both beneficial insects and herbivores (Paré and Tumlinson, 1999; Pichersky and Gershenzon, 2002; Dudareva et al., 2006). In addition, VOCs are also involved in indirect defence by attracting natural enemies of herbivores (i.e., predators and parasitoids) towards the damaged sites (De Boer et al., 2004; McCormick et al., 2012). Furthermore, they can also mediate within-plant signalling (Frost et al., 2007; Heil, 2010) and between plant communication (Karban et al., 2000; Kessler et al., 2006), and affect the composition of root, floral and foliar microbiota (Stotzky et al., 1976; Junker and Tholl, 2013). VOCs can mediate multiple co-occurring interactions (Dicke et al., 2009; Ponzio et al., 2013), both above- and below-ground (Bezemer and van Dam, 2005), making them a major currency for plant communication.

VOCs vary among plant species, cultivars, varieties, genotypes and tissues within the same plant (Krips et al., 2001; Jönsson et al., 2005; Kappers et al., 2011) and in response to biotic and abiotic factors, e.g. herbivory or drought (Holopainen and Gershenzon, 2010; McCormick et al., 2016). These properties make VOCs a good source of information about the identity and status of the emitting plant to other organisms. Considering the multiplicity and complexity of volatile-mediated interactions, we argue that VOCs can play an essential role in determining the outcome of competition in plant communities. In the following section, we illustrate some of the potential ecological roles of plant volatiles in competition, which we summarise in Fig. 2.

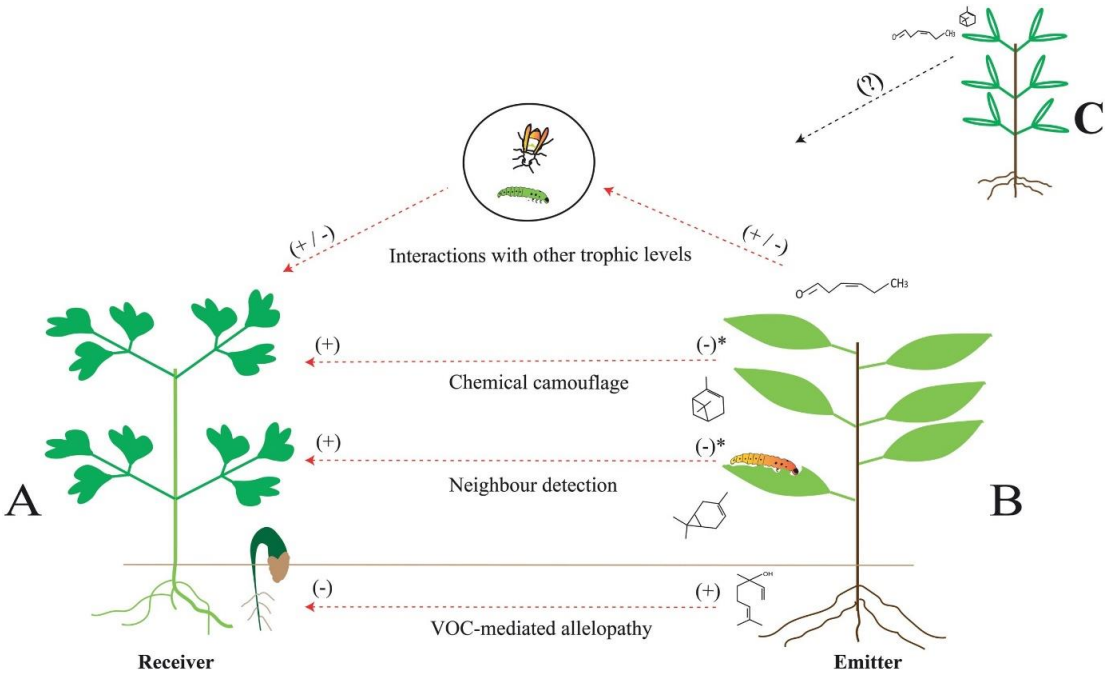

Figure 2. Volatile-mediated interactions and their potential influence on competition outcomes in plant communities. Dashed lines indicate interactions and arrowheads show the direction of the interactions. Signs indicate the expected positive (+) and negative (−) effects for the receiver and emitter, while question marks (?) indicate unknown effects that require further investigation. Asterisks (*) indicate that although there may not be an immediate disadvantage for the emitter, there could be one in the future if the receiving plant becomes a better competitor by the use of the cues. In this example, A is the receiver plant, and B is the emitter, but similar interactions can occur in the opposite direction. C Indicates other plant species within the same community.

2.2 Potential roles of VOCs in plant competition

2.2.1 Modifying interactions with other trophic levels

As mentioned earlier, plant volatiles serve multiple roles in plant-arthropod interactions. Therefore, one potential function of VOCs in plant competition would be to enhance the emitter's competitive ability by modifying these

interactions. For instance, it has been shown that volatiles released by different barley (*Hordeum vulgare*) varieties in competition scenarios can reduce the acceptability of the receivers for the Birdcherry-oat aphid, *Rhopalosiphum padi* (Pettersson et al., 1999; Ninkovic et al., 2002). Likewise, in the case of red clover (*Trifolium pratense*), the total emission of herbivore-induced volatiles, mainly terpenoids, was higher in plants grown with heterospecifics, compared to those growing with conspecifics or alone (Kigathi et al., 2013). Terpenoids are well known to mediate the attraction of natural enemies of herbivores, both above- and below-ground in other systems (Kappers et al., 2005; Rasmann et al., 2005; Hiltpold et al., 2010). These examples support the notion that the emitter plant can obtain indirect benefits by reducing herbivory and enhancing the recruitment of natural enemies. Whether or not there is a benefit associated with other interactions impacting plant reproduction and health (e.g., those with pollinators, seed dispersers, and beneficial microorganisms) requires further study.

There may be some advantages for the receiver plant as well, as certain plant associations may decrease the likelihood of detection or vulnerability to herbivores, leading to "associational resistance" (Barbosa et al., 2009), in which VOCs can also be involved. For example, *Galerucella* spp. leaf beetles specialise either on *Filipendula* sp. or on *Lythrum* sp., and have a common hymenopteran parasitoid *Asecodes mento*. Stenberg et al. (2007) found that *A. mento* preferred herbivore-induced VOCs emitted by *Filipendula* sp. and that parasitization rates were higher on *Galerucella* beetles specialised on this plant species. However, mixed populations of *Filipendula* and *Lythrum* supported higher densities of

their shared 'bodyguard' than monospecific *Filipendula* populations, presumably due to the increased attractiveness of the volatile mix. This indicates that there may be associational resistance, and that the competing species producing the least attractive volatile blend (*Lythrum*) could benefit from it. A similar scenario can occur in intraspecific competition, as reported by Zakir et al. (2013), who found significant reduction in oviposition by *Spodoptera littoralis* females on undamaged cotton plants (*Gossypium hirsutum*) adjacent to damaged conspecifics only having above-ground contact (Zakir et al., 2013), as gravid females avoid ovipositing on or nearby damaged plants.

Considering the complexity of interactions mediated by volatile compounds there could also be adverse effects at both the emitting and the receiving ends, e.g., "associational susceptibility" by increasing the likelihood of detection or vulnerability to herbivores (Barbosa et al., 2009), or the attraction of other antagonistic organisms e.g., hyperparasitoids, eavesdropping on these cues (Dicke and Baldwin, 2010). However, to date, there is little experimental evidence exploring those indirect negative effects in plant competition.

2.2.2 VOC-mediated allelopathy

Another role of plant volatiles benefiting the emitter is allelopathy. The release of chemicals influencing growth or germination of other plants, is perhaps one of the best described and well-studied phenomena involving plant secondary metabolites (Einhellig, 1995; Kruse et al., 2000; Romagni et al., 2000; Inderjit and Duke, 2003), and has been widely exploited in agricultural settings (Olofsdotter et al., 2002; Weston and Duke, 2003; Khan et al., 2008). Most

studies focus on non-volatile compounds released from plant roots such as flavonoids, phenolics, enzymes, and organic acids (reviewed in Inderjit and Duke, 2003) but some evidence suggests that volatile organic compounds emitted above- and below-ground by plants can confer a competitive advantage to the emitter due to their allelopathic properties.

In a laboratory test, volatile compounds β-terpineol, linalool, eugenol and tetradecanoic acid emitted by tomato (*Solanum lycopersicon*) foliage inhibited seed germination of the tropical plant *Amaranthus mangostanus* in a concentration-dependent manner (Kim and Kil, 2001). Likewise, exposure of lettuce (*Lactuca sativa*), barnyard grass (*Echinochloa crus-galli*), and wheat (*Triticum aestivum* cv. Grana) to volatile substances released from pulverised leaves of some Brassicaceae species, particularly *Brassica nigra* and *B. juncea*, resulted in delayed germination and reduced growth (Oleszek, 1987). This was attributed to the presence of degradation products of glucosinolates, which are known for their allelopathic properties. However, some plant species emit volatiles having allelopathic effects against glucosinolate-producing plants. For example, monoterpenes camphor, 1,8-cineole, and β-pinene emitted by purple sage (*Salvia leucophylla*), inhibited germination and root growth of field mustard, *Brassica rapa* (Nishida et al., 2005).

In addition to VOCs released from aboveground organs, root volatiles can also be detrimental to the development of neighbouring plants. For example, big sagebrush (*Artemisia tridentata*) root volatiles inhibited seed germination of wild tobacco (*Nicotiana attenuata*). When individual compounds from the

volatile-blend were tested, methyl jasmonate inhibited seed germination of *N. attenuata* at all tested doses, while the phytotoxicities of 1,8-cineol, camphor, nerol, α-thujone, and geraniol were dose-dependent (Jassbi et al., 2010). Other authors including Viles and Reese (1996) and Sun et al. (2011) have also reported the allelopathic properties of volatiles emitted from the roots of different plants.

Leaf litter, as observed in terpene-storing species, can be an important source of VOCs (Kainulainen and Holopainen, 2002; Inderjit et al., 2011), and these compounds are known to have detrimental effects on competitors. For example, exposure to leaf litter aqueous extracts of Sydney blue gum (*Eucalyptus saligna*) inhibited germination and initial growth, while increasing H_2O_2 levels and electrolyte leakage of the seedling membrane of recipient plants (Silva et al., 2017). Similarly, VOCs from both fresh and dry leaves of two Brazilian plants (*Heterothalamus psiadioides* and *Baccharis patens*) inhibited seed germination and affected shoot and root elongation in the receiver plants (Silva et al., 2014). Furthermore, in some plant species (e.g., conifers), leaf litter VOCs can persist over a long period of time and be released in detectable concentrations even after several months of decomposition on the soil surface (Kainulainen and Holopainen, 2002).

2.2.3 Neighbour detection

Receiving plants could use VOCs to identify possible competitors and respond accordingly. For instance, ethylene, which is a ubiquitous volatile phytohormone, induces the development of shade avoidance traits such as

increased leaf angles and stem elongation in tobacco plants (*Nicotiana tabacum*); while transgenic tobacco plants insensitive to ethylene delayed the development of these traits, making them less competitive than their ethylene-sensitive wild-type neighbours (Pierik et al., 2003). It has been hypothesised that plants use volatile cues from their neighbours to monitor and predict potential competitors and prepare physiologically for future competition (Ninkovic et al., 2013, 2016).

At a higher level of complexity, plants release different volatile blends in response to their physiological/phenological modifications, biotic stress (e.g., herbivore or pathogen attack) and the ever-changing environment (McCormick, 2016), making them ideal sources of more detailed information about the identity and state of the emitter. Therefore, neighbouring plants could eavesdrop on the emitters "volatile-coded information" and in response, allocate resources to organs required for competition or defence, giving them a competitive advantage. Below, we illustrate some examples of how plants could use information on neighbour identity and state in competition.

2.2.3.1 Establishing neighbour identity

The ability of organisms to recognise their relatives could lead to the exhibition of different behaviours between conspecifics due to their genetic relatedness (Gamboa et al., 1991). Different organisms including mammals (Mateo, 2002), birds (Krause et al., 2012), insects (Lihoreau et al., 2007) and microbes (Mehdiabadi et al., 2006) have demonstrated kin recognition, and there is

evidence showing that plants can also do this (Dudley and File, 2007; Murphy and Dudley, 2009; Biedrzycki et al., 2010).

Consequent to their exposure to neighbours' VOCs, plants may alter their biomass allocation to specific organs depending on how close the interacting species relates genetically, which can also be viewed as preparedness for competition. For instance, barley cultivar Kara (*Hordeum vulgare*) allocates more biomass to roots when exposed to the volatiles of different cultivar (Alva) compared to volatiles of the same cultivar or to clean air (Ninkovic, 2003). Overall, total biomass was not affected by whether Kara was exposed to clean air, Alva volatiles, or other Kara volatiles. However, Kara plants exposed to Alva volatiles had an increase in specific leaf area, which could be indicative of readiness for light competition.

In addition to biomass allocation, volatiles from conspecifics and heterospecifics can also affect direct and indirect defences. Sagebrush plants (*Artemisia tridentata*) that received volatiles from genetically identical damaged plants increased their herbivore resistance, compared to those that received volatiles from non-self-cuttings. These findings were confirmed by microsatellite markers, both under laboratory and field conditions (Karban and Shiojiri, 2009; Karban et al., 2013). Likewise, red clover (*Trifolium pratense*) significantly reduced the emission of herbivore-induced volatiles when growing with conspecifics, as opposed to when growing with heterospecifics (*Dactylis glomerata*) (Kigathi et al., 2013). This suppression was observed when plants had contact above- ground only, below-ground only, and both above- and below-

ground, evidencing that kin recognition is crucial in volatile-mediated competition between plants, both above- and below-ground.

Recent studies indicate that plants can also respond to population and gender differences in their neighbours. Studies on sagebrush (*Artemisia tridentata*) (Karban et al., 2016) and lima bean (*Phaseolus lunatus*) (Moreira et al., 2016) suggest that plants have "population dialects", where members of the same population are more efficient at producing anti-herbivore defences upon exposure to VOCs from damaged plants. Another study reported that dioecious plants *Baccharis salicifolia* can distinguish the gender of their neighbours based on volatile cues, with male emitters inducing herbivore resistance in both male and female receivers, whereas female emitters only induced resistance on same gender plants (Moreira and Abdala-Roberts, 2018). These examples illustrate the multiple layers of information conveyed by plant volatiles and their potential roles in plant competition.

2.2.3.2 Establishing neighbour state

Upon attack, plants release a unique volatile blend that differs from that of undamaged plants (Walling, 2000; Huang et al., 2003; Hare, 2011). Herbivore-induced plant volatiles are involved in both direct and indirect plant defence by deterring or repelling herbivores and recruiting their natural enemies (Kessler and Baldwin, 2001; Heil, 2008; McCormick et al., 2012, 2016).

Healthy, undamaged plants can recognise this "induced blend" from their neighbours and undergo physiological changes that will enhance their readiness

to future attack. This physiological process by which a plant prepares to more quickly or aggressively respond to future biotic or abiotic stress is known as "priming", and the condition of readiness is termed "primed state" (Frost et al., 2008). For instance, *Nicotiana attenuata* exposed to the VOCs from clipped *Artemisia tridentata* accelerated its production of trypsin proteinase inhibitors (primed state), which in turn resulted in lower herbivore damage by *Manduca sexta* and higher mortality rate of young *M. sexta* caterpillars (Kessler et al., 2006). Also, Ton et al. (2007) exposed undamaged maize plants to volatiles from *Spodoptera littoralis*-wounded conspecifics, and reported an up-regulation in defence-related gene expression and changes in VOC emissions (primed state). *S. littoralis* caterpillars that fed on primed maize had lower growth rates compared to those that fed on healthy un-primed plants, and the parasitic wasp *Cotesia marginiventris* showed a preference towards the VOC blend emitted by primed plants versus that of healthy un-primed ones (Ton et al., 2007). Another study by Frost et al. (2007), using hybrid poplar (*Populus deltoides* x nigra), showed that saplings also enhanced their defence state when exposed to herbivore-induced plant volatiles (Frost et al., 2007), suggesting that priming is not restricted to annual herbaceous plants.

Priming does not confer resistance per se, but rather allows accelerated induced resistance once an attack occurs (Frost et al., 2008). However, it could impact competition outcomes if the receiver plant would gain resistance at a lower cost relative to the induction of direct or indirect defences if not primed (van Hulten et al., 2006), allocating more resources to competition in this primed state. It

would be of interest to explore if receiver plants incur fitness costs if not attacked, due to resource trade-offs favouring defence at the expense of growth and reproduction (Jing and Coley, 1990; Baldwin et al., 1998; Fine et al., 2006).

2.2.4 Chemical camouflage

A fascinating, yet less explored possibility is that receiver plants use "environmentally acquired chemical camouflage" (Kessler and Kalske, 2018) in competition scenarios to decrease their appearance to herbivores. Bidirectional exchange of highly volatile compounds such as monoterpenes between plants and the atmosphere can occur through the leaf stomata (Niinemets et al., 2014). If plants can adsorb and re-release compounds from other plants, then, these could affect plant communication and multitrophic interactions (Mofikoya et al., 2017). An example illustrating this possibility is a study by Himanen et al. (2010), where authors reported that under laboratory, semi-field and field settings, the leaves of birch (*Betula* spp.) adsorb and re-release volatiles produced by marsh Labrador tea (*Rhododendron tomentosum*), thereby reducing their attractiveness to herbivores (Himanen et al., 2010). In a follow-up experiment, broccoli (*Brassica oleracea*) was also able to adsorb and re-release volatiles from *R. tomentosum* becoming less susceptible to *Plutella xylostella* oviposition and herbivory (Himanen et al., 2015), thus indicating that the phenomenon may be widespread in the plant kingdom. It is conceivable that chemical camouflage may increase the fitness of the plant adsorbing the volatile products of the neighbour due to reduced herbivory and a lower energy investment in producing the compounds itself.

From the examples above, it can be argued that there is overlap between the mechanisms involved in volatile-mediated plant-plant interactions. We have attempted to typify different mechanisms, but it is important to highlight that these are not mutually exclusive and may be part of a continuum, rather than being discrete events. Also, it is important to note that their occurrence is likely to depend on multiple factors including the species competing, their phenological and physiological state, and extrinsic biotic and abiotic factors.

2.3 Conclusions and future outlook

The use of morphological traits, growth, and total biomass in plant competition studies, may be insufficient to understand the role of complex community interactions. Plant VOCs mediate multiple ecological networks, and they may influence competitive outcomes in favour of the plant that is best adapted to exploit them. It is important to emphasise that measuring volatile emissions alone is not enough to understand their full impact on ecological interactions. It only provides meaningful information when combined with relevant data on the direct or indirect benefits or disadvantages associated with volatiles to the emitting or receiving plants, e.g., direct effects on health, growth, or reproduction; or indirect effects by modifying the behaviour of animals or microorganisms, that have a measurable impact on plant fitness. This requires a multidisciplinary approach that incorporates fields such as plant physiology, biochemistry, ecology, molecular biology, microbiology, and entomology among others; along with laboratory and field studies to investigate changes at

the emitting and receiving ends, the mechanisms behind individual interactions, and how these coexist and operate under natural conditions.

Many of the papers cited here provide innovative experimental designs to isolate the effect of volatiles from those of other environmental variables under controlled and semi-controlled conditions (e.g., restricting below-ground contact, or using dispensers to release natural blends, synthetic blends or compounds of interest). Many of these strategies could be implemented to test the allelopathic effects of volatiles on the growth, phenology, and reproduction of competing plants in laboratory settings. More complex experimental designs (e.g., semi-field experiments) can be used to evaluate whether different plant pairings or associations affect either the attractiveness or resistance of both emitting and receiving plants to herbivores; their ability to lure natural enemies and other beneficial insects, and the net outcome of these interactions in terms of plant growth, health, and reproductive success. To test the underlying mechanisms of priming, we must utilise to molecular techniques to establish which particular genes or pathways are upregulated or downregulated by exposure to herbivore-induced volatiles from neighbouring plants in a competitive context and follow up with relevant measurements and bioassays to test the ecological significance of these findings. Chemical camouflage could be experimentally investigated, for example, by using pathway-specific inhibitors (e.g., Fosfidomycin or Lovastatin that inhibit terpene biosynthesis) (Junker et al., 2011) and then exposing test plants to the compounds of interest (whose production has been inhibited) in a range of concentrations. Then the ability of

the receiver plants to adsorb and re-release those compounds can be tested, and further experiments can be used to evaluate the success of these plants in competition scenarios. The use of mutants, transgenics, and natural variants can further aid in elucidating the underlying mechanism behind different competitive strategies (Pierik et al., 2003; Pierik et al., 2004).

For future studies in the field, we suggest measuring VOCs in addition to morphological parameters, together with samples from the surrounding plants, arthropod and microbial communities, as well as environmental and climatic data, and monitoring changes over time. Although this approach does not make it possible to establish direct causal relationships between VOCs and plant responses, this does not prevent researchers from drawing ecologically relevant conclusions from their data resulting in powerful insights into the ecological chess game of plant competition. Dealing with complex data sets and the uncertainty of causal relationships is and has always been a challenge for ecologists, but there are multiple statistical and modelling tools to assist researchers in this task (Gauch and Gauch, 1982; Lepš and Šmilauer, 2003; Legendre and Legendre, 2012).

One of the main limitations in the study of plant VOCs in competition is the lack of understanding of the exact mechanisms by which plants perceive exogenous volatiles, i.e., the organs, tissues, and cells involved in plant ‘olfaction’. Further research in this field promises to yield exciting results in the future. It has been proposed that the exchange of volatile compounds between the atmosphere and plants can be dependent on stomatal conductance (Niinemets et al., 2014) and

evidence suggests that the mechanisms by which plants respond to exogenous plant volatiles can be both passive and active. These passive mechanisms may involve the adsorption and re-releasing of neighbour's VOCs (Choh et al., 2004; Himanen et al., 2010), while active responses could include changes in gene expression in the receiver plant in response to VOCs (Zhang et al., 2012; Ye et al., 2018). Although the general patterns are clear, their molecular mechanisms remain unknown. Induced plant responses to herbivore and pathogen attack are well understood at the molecular level (Kombrink and Somssich, 1995; Dangl and Jones, 2001; Kessler and Baldwin, 2002; Arimura et al., 2009). However, there is still much that needs to be investigated about the plant's responses to VOCs from neighbouring plants, including the identity of the elicitors, early and secondary signal transduction pathways, and the role of phytohormones. After these questions are answered, other questions related to signal stability, specificity, and action range are sure to follow.

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Chapter 3

Natural variation in volatile emissions of the invasive weed *Calluna vulgaris* in New Zealand

Native plants stand a little chance, where heather is prolific.
Photo credit: ***Paul G. Peterson*** *(Manaaki Whenua) and* ***Paul D. Barrett*** *(Massey University).*

This chapter investigates the volatile emissions of *Calluna vulgaris* growing at different sites on the Central North plateau and explores the biotic and abiotic factors contributing to emissions. The chapter is presented in the style of the journal *Plants* with minor structural modifications. The chapter was published by the journal *Plants* as:

Effah, E., Barrett, D.P., Peterson, P.G., Godfrey, A.J.R., Potter, M.A., Holopainen, J.K. and Clavijo McCormick, A., 2020. Natural Variation in Volatile Emissions of the Invasive Weed *Calluna vulgaris* in New Zealand. *Plants* 9, 283.

Abstract

Invasive plants pose a threat to natural ecosystems, changing the community composition and ecological dynamics. One aspect that has received little attention is the production and emission of volatile organic compounds (VOCs) by invasive plants. Investigating VOCs is important because they are involved in vital ecological interactions such as pollination, herbivory and plant competition. Heather, *Calluna vulgaris*, is a major invasive weed in New Zealand, especially on the Central Plateau, where it has spread rapidly since its introduction in 1912, outcompeting native species. However, the chemical behaviour of heather in its invaded ranges is poorly understood. We aimed to explore the natural variation in volatile emissions of heather and the biotic and abiotic factors influencing them on the Central Plateau of New Zealand. To this end, foliar volatiles produced by heather at four different sites were collected and analysed using gas chromatography coupled to mass spectrometry. Soil properties, herbivory and other environmental data were also collected at each site to investigate their effects on VOC emissions using generalised linear models (GLMs). Our results reveal significant differences in VOC emissions between sites and suggest that soil nutrients are the main factor accounting for these differences. Herbivory and temperature had only a minor effect, while soil

water content had no impact. Further studies are needed to investigate how these variations in the invasive plant's foliar volatiles influence native species.

Keywords: Heather, Invasive species, Plant scents, Plant secondary metabolites, Soil nutrients, Volatile organic compounds

3.1 Introduction

The intentional or accidental introduction of exotic species, including plants, into new regions, poses a threat to biodiversity [1]. Plant invasions have escalated in recent times, mainly because of increased human migration, global trade, and climate change [2-4]. Many morphological, physiological and reproductive traits associated with invasiveness in plants have been explored to understand and mitigate negative impacts [5]. Chemical research has focused on the allelopathic properties of exudates (fluids) released by invasive plants [6-9], while much less is known about volatile organic compounds (VOCs)-scents- and their ecological impacts [10]. VOCs are the main currency in plant communication, mediating multiple interactions between the emitting plant and other organisms including beneficial arthropods (such as pollinators and seed dispersers), herbivores, natural enemies of herbivores, microorganisms (e.g., mycorrhizae), pathogens and other plants [11].

Plant volatile emissions are species-specific [12] but also plastic to the changing environment, and are known to change in response to biotic variables such as herbivory and pathogen attack, and abiotic variables such as temperature, soil nutrients, and ultraviolet-B radiation (UV-B), among others [13]. Previous

studies suggest that VOCs emitted by invasive species can inhibit seed germination and reduce the above and below-ground growth of nearby plants, with direct benefits to the emitting plant [14-17]. Due to their ecological value and allelopathic effects, VOCs can be considered valuable "weapons" in plant competition [10, 18]. Given the relevance of plant volatiles, the aim of this study is to characterise the natural variation in VOC emissions of an invasive species in the field and explore the biotic and abiotic factors contributing to this variation.

The Central Plateau is a volcanic area covering the central part of the North Island of New Zealand, including the Tongariro Natural Park (TNP), a world heritage site of natural and cultural value. Common native plants in this area include *Chionochloa rubra* (red tussock) and *Poa colensoi* (blue tussock) (both grasses), and *Leptospermum scoparium* (mānuka) and *Dracophyllum subulatum* (*Dracophyllum*) (both woody perennial species) [19]. *Calluna vulgaris* (heather) is a European native perennial shrub from the family Ericaceae. This alien species was deliberately introduced to the Central Plateau in 1912 [20, 21] and is now the most widespread invasive weed in this area, covering more than 50,000 ha of the TNP and surrounding land (Figure 1), representing the most substantial infestation of heather in New Zealand.

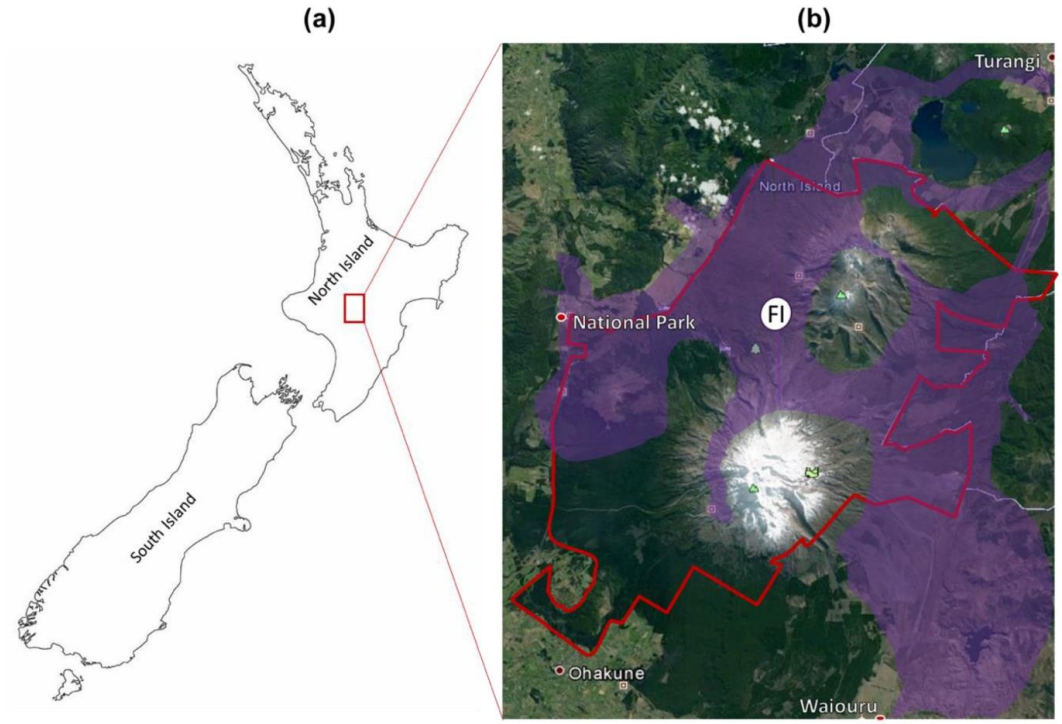

Figure 1. The distribution of heather in the Central North Island. (**a**) North and South Islands of New Zealand. (**b**) Invasion of heather in the Central Plateau. Heather was first planted in the white region (FI) but has now spread through all the regions in purple. The boundary of Tongariro National Park (TNP) is shown in red.

On the Central Plateau, heather has invaded the seral tussock grasslands, modifying soil properties, outcompeting native vegetation and disrupting the natural processes of plant regeneration and succession [22-25]. Phytophagous insect diversity and abundance are also negatively affected by heather invasion due to the changing habitat, loss or reduction of native food plants and increased arachnid predation [22]. Efforts to control this species, including the use of herbicides and introduction of a Chrysomelid biocontrol agent *Lochmaea suturalis*, have been of limited success due to the persistence of seedbank in the soil and poor establishment of the biocontrol agent [23, 26].

A recent study explored the VOC emissions of heather as a main component of heath ecosystems in its native range (Denmark) [27] and found this species to be a rich terpenoid emitter, reporting at least 15 monoterpenes and the homoterpene (*E*)-4,8-Dimethyl-1,3,7-nonatriene. The authors investigated seasonal variation and emission responses to six years of climatic manipulations (elevated CO_2, extended summer drought and night-time warming) in a semi-natural setting and found seasonal variation in VOC emission but also a significant effect of the abiotic variables tested. However, to our knowledge, our study is the first to explore the VOC emissions of heather in its invasive range and the factors affecting their emissions under natural conditions (without experimental manipulation).

3.2 Materials and Methods

3.2.1 Study Area

The study was conducted during the summer of 2017 – 2018, under natural conditions on the Central Plateau of the North Island, New Zealand. The region has a mean daily temperature of 12 – 13° C in summer and 9 – 10° C in winter, with low-fertility soils formed predominantly from volcanic ash [19, 21, 28]. Four sites (about 561 m^2 per site) were set up: three within the Waiouru Military Training Area (WMTA) to the east of TNP and the fourth site near Erua, a small settlement on the western border of TNP (Appendix 1.1).

3.2.2 Sampling of Volatiles

Five plants of the same size and phenology were selected at each of the four sites.

The introduced heather beetle (*Lochmaea suturalis*) has been released into this region as a biocontrol agent [26], but it is patchily distributed; therefore, we deliberately avoided sites where the beetle was present to reduce variation in herbivory within sites. Foliar volatile samples from heather were collected at each of the four sites. VOCs were sampled by using the "push–pull" headspace sampling technique [29]. Similar amounts of foliage from each sampled plant were enclosed in new multi-purpose cooking bags (AWZ Products, 50 cm × 30 cm) with their ends fastened. Using a portable PVAS22 pump (Volatile Assay Systems Rensselaer NY), carbon-filtered air was pushed into the bags through a PTFE tube (1.70 L/min) and simultaneously pulled out through another tube (1.20 L/min).

To collect the VOCs, a volatile collection trap with 30 mg HayeSep Q adsorbent (Volatile Assay Systems Rensselaer, NY, USA) was inserted in the pull tube [30, 31]. Collections for each target plant were done for two hours during stable environmental conditions, over a period of four days in early December prior to heather flowering [32]. To control for collection time, random samples were collected simultaneously from different sites, and these were pooled for final analysis for each site. After VOC sampling, the foliage enclosed in the bags was excised and collected to measure herbivore damage. Plant material was subsequently oven-dried at 60° C for 72 h and weighed (3-digit accuracy) to estimate VOC emissions per dry weight (grams).

Collection filters were eluted using 200 µL of 95% hexane with 10 ng/mL nonyl acetate ($C_{11}H_{22}O_2$) (Sigma Aldrich) as an internal standard. The samples were

analysed using gas chromatography coupled to mass spectrometry (Shimadzu technologies) with a 30 m × 250 µm × 0.25 µm TG-5MS column and helium as a carrier gas. Operating conditions were as follows: injector temperature 230° C; split ratio of 10 : 1; initial oven temperature at 50° C, held for 3 min then increased to 95° C at a rate of 5° C/min. Tentative identification of compounds was achieved by comparing them with target spectra in the MS library from the National Institute of Standards and Technology (NIST) and, when available, verified by authentic standards (Sigma Aldrich). Contaminants were excluded from analyses by identifying compounds in blank (air in empty cooking bags).

3.2.3 Soil Sampling

To determine soil properties, four soil cores (15 cm deep × 3 cm diameter) were collected at random points surrounding each sampled plant, for a total of 20 soil cores per site. The fresh weight of each sample was measured on the day of collection, and then oven-dried (40° C) to constant weight. Soil water content (SWC) was measured gravimetrically [33] and expressed as a percentage. After estimating SWC, cores collected from each site were homogenised and used for soil nutrient analysis (as averages for respective sites). Soil pH, Olsen phosphorus, potassium, calcium, magnesium, sodium, organic matter, total carbon and nitrogen were analysed by R. J. Hill Laboratories Limited, Hamilton–New Zealand.

3.2.4 Ambient and Soil Temperature Measurements

The ambient temperatures for experimental sites were obtained by installing

temperature data loggers (Tinytag, Gemini) from mid-November to mid-December 2017. Soil temperatures were taken from five positions covering each site using a soil temperature probe.

3.2.5 Arthropods on Heather

Arthropods were collected from each sampled plant using the beating tray technique [34, 35]. Beating of a branch on a tray was done immediately after volatile collection, but on an adjacent branch as the branch used for VOC collection was excised. Collected specimens were preserved in 70% ethanol and identified to order.

3.2.6 Herbivore Damage on Heather

The foliage enclosed in the bags from VOCs sampling was used to estimate herbivore damage. Due to the small size of the leaves, visible herbivore damage was assessed using a handheld magnifying glass. The number of damage marks seen on foliage was counted as illustrated in Figure 6a. To eliminate the bias of damage count being correlated with foliage size, the number of damage marks was divided by the dry weight (DW) of the respective foliage (i.e., herbivory per DW).

3.2.7 Data Analysis

Statistical analyses were performed using RStudio, Version 1.1.456 (RStudio: integrated development for R) [36]. The Shapiro-Wilk test was used to check the normality of herbivory, arthropod counts, and abiotic variable data; then, these were analysed using either analysis of variance (ANOVA) or a non-

parametric Kruskal–Wallis test. When significant differences were found, Tukey's honestly significant difference (Tukey's HSD) or Mann–Whitney pairwise tests were used for post-hoc comparisons.

Principal component analysis (PCA) was performed using all the volatile compounds identified from the headspace of heather. PCA and descriptions of variable presentations in respective components were performed using the "FactoMiner" package [37]. A Generalised Linear Model (GLM) assuming Gamma distribution (log-link) was first performed to compare the proportions of VOC classes between the four sites using the GLM function in R. VOC classes were response variables while the four study sites were used as a categorical predictor. The relevel function was used to construct a set of level contrasts for the four sites [38, 39] and the Wald test used to evaluate the significance of estimated effects [40].

A second GLM was then performed to determine the effect of environmental variables on volatiles emitted by heather. VOCs with higher contributions in PCA were the response variables. Herbivore damage, soil water content (SWC), average daytime temperature and primary macronutrients (nitrogen, phosphorus and potassium) were initially selected as potential predictor variables. These predictors were selected based on their importance to plant performance and VOC emissions [13, 41-43]. To reduce collinearity, we performed a pairwise correlation between all predictor variables (Appendix 1.2), and those with high correlation were removed based on how they correlated with other variables [44]. This resulted in keeping only herbivory, ambient temperature, SWC, and

potassium (as a proxy for nutrients other than nitrogen, which was strongly correlated with soil water content) in the final model, and all continuous predictor variables standardised (z-scores) prior to modelling [44]. In all the GLMs performed in this paper, we added a small constant (0.001) to all response variables to avoid the problem caused by expected values coming out as zero. This value was arbitrarily chosen but much smaller than the minimum observed emission rates for all the response variables and was tested for sensitivity to minimise the risk of contaminating findings while ensuring model convergence.

3.3 Results

3.3.1.1 Volatile Emissions by Heather

After collecting samples from the headspace (i.e., surrounding airspace) of heather branches enclosed in nylon cooking bags and analysing them using a Gas Chromatograph-Mass Spectrometer (GC-MS), we identified 33 volatile compounds and grouped them under their respective chemical classes (Appendix 1.3). The most abundant compounds were fatty acid derivatives (33.3%), monoterpenes (21.2%) and sesquiterpenes (33.3%). Of the 33 VOCs identified from heather, a typical fungal volatile 1-octen-3-ol [45] was among the most abundant compounds at all sites (Appendix 1.3).

A comparison between sites revealed that total volatile emissions were significantly lower at site 4 when compared to site 1 (GLM; $\beta = -1.32$, $X^2 = 5.66$, $p = 0.017$) and site 2 (GLM; $\beta = -1.14$, $X^2 = 4.27$, $p = 0.039$). The same was true for homoterpenes, sesquiterpenes and fatty acid derivatives (Figure 2). Total fatty acid derivatives were significantly lower at site 4 compared to

site 1 (GLM; β = −1.34, X^2 = 5.30, p = 0.021) and site 2 (GLM; β = −1.15, X^2 = 3.88, p = 0.049). Total sesquiterpenes were also significantly lower at site 4 compared to site 1 (GLM; β = −1.75, X^2 = 13.20, p < 0.001), site 2 (GLM; β = −1.68, X^2 = 12.0, p = 0.001) and site 3 (GLM; β = 1.25, X^2 = 6.72, p = 0.010). The proportion of homoterpenes was significantly higher at site 1 (GLM; β = 6.03, X^2 = 114.30, p < 0.001), site 2 (GLM; β = 5.24, X^2 = 86.10, p < 0.001) and site 3 (GLM; β = 4.53, X^2 = 64.50, p < 0.001) compared to site 4.

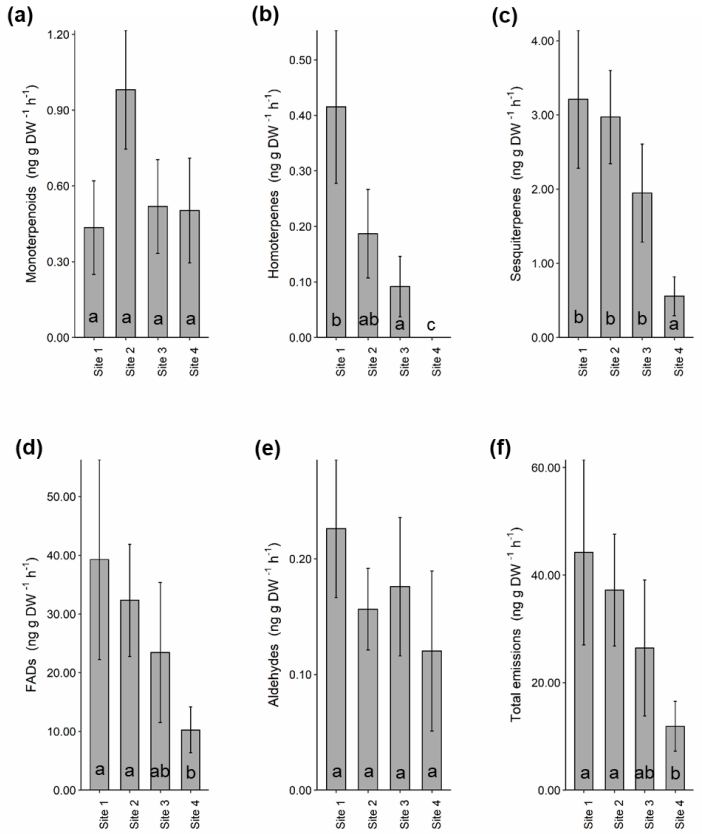

Figure 2. Volatile organic compound (VOC) classes identified from the headspace of heather at four different sites. Bars show mean ± SE of total (**a**) monoterpenoids, (**b**) homoterpenes, (**c**) sesquiterpenes, (**d**) fatty acid derivatives, (**e**) aldehydes and (**f**) total volatile emissions measured from target plants from each site (n = 5). Letters indicate pairwise comparisons between sites. Abbreviations: fatty acid derivatives (FADs).

3.3.1.2 Principal Component Analysis Based on Volatiles Emissions at Different Sites

We used principal component analysis (PCA) to further explore differences in plant volatile emission between sites. The first axis of principal component analysis (PC1) explained about 31% of the total variance in VOC emissions among the four sites and was mostly characterised by fatty acid derivatives and (*E*)-4,8-Dimethyl-1,3,7-nonatriene ((*E*)-DMNT) (Figure 3). PC2 was characterised by sesquiterpenes and explained about 13% of the variability. The first six principal components (PC1 to PC 6) captured over 78% of the variability in the data and were considered in subsequent analyses. Sites 1 and 4 were clearly separated from one another based on VOC emitted by heather at these sites, while sites 1, 2 and 3 overlapped.

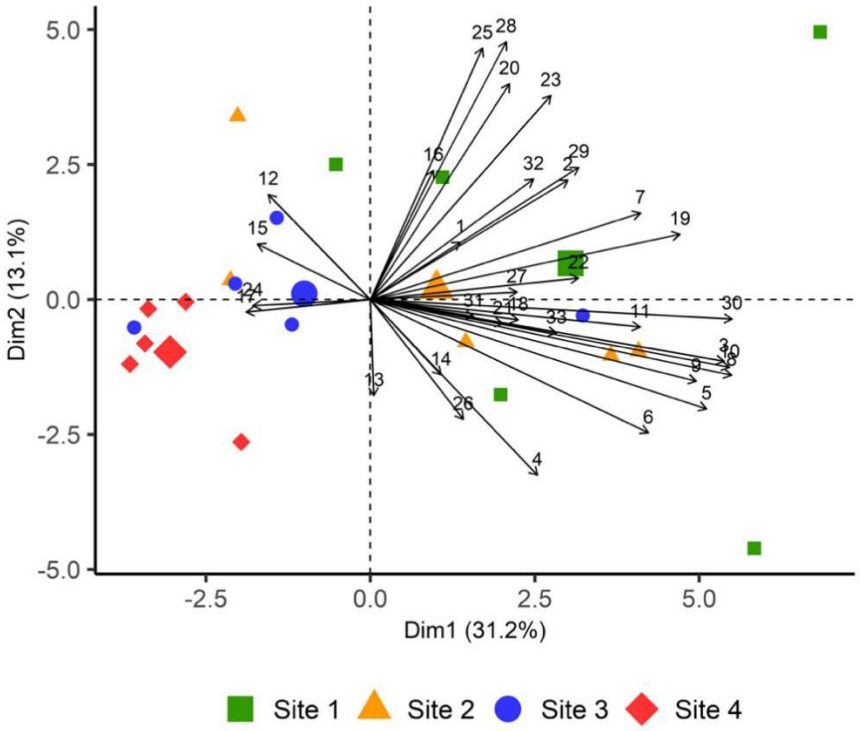

Figure 3. Principal components analysis (PCA) biplot showing PC scores of individuals and loadings of variables. PCA was based on 33 VOCs emitted by heather from all sites. Large symbols indicate centroids for respective group. The numbers in the graph indicate the following compounds; (1) hexyl acetate, (2) 1-hexanol, (3) (*Z*)-2-hexenol, (4) (*Z*)-3-hexenol, (5) (*Z*)-3-hexenyl 2-methylbutyrate, (6) (*Z*)-3-hexenyl acetate, (7) (*Z*)-3-hexenyl benzoate, (8) (*Z*)-3-hexenyl butyrate, (9) (*Z*)-3-hexenyl hexanoate, (10) (*Z*)-3-hexenyl isobutyrate, (11) (*Z*)-3-hexenyl valerate, (12) α-pinene, (13) α-terpineol, (14) β-myrcene, (15) β-pinene, (16) limonene, (17) linalool, (18) (*Z*)-β-ocimene, (19) (*E*,*E*)-α-farnesene, (20) α-gurjunene, (21) (*E*)-β-caryophellene, (22) δ-cadinene, (23) γ-elemene, (24) copaene, (25) germacrene B, (26) germacrene D, (27) humulene, (28) (*E*)-β-famesene, (29) (*Z*,*E*)-α-farnesene, (30) (*E*)-4,8-Dimethyl-1,3,7-nonatriene, (31) 1-octen-3-ol, (32) decanal, (33) nonanal. Compounds were assigned to the following classes: fatty acid derivatives (1 – 11), monoterpenes (12 – 18), sesquiterpenes (19 – 29), homoterpene (30), alcohol (31), aldehydes (32 -33).

3.3.2 Soil Nutrients and Environmental Variables

We collected soil samples and environmental data (daytime temperature, soil water content and soil temperature) at each site. Soils analyses revealed that all sites were nutrient poor; however, at site 4, most nutrients, except for N, were lower (Table 1).

Table 1. Comparison of soil properties between experimental sites. The medium or optimum range guidelines relate to Hills' laboratories' crop guides for mixed pasture.

Soil properties	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Reference (Medium Range)**
Total nitrogen (%)	0.19	0.30	0.17	0.26	0.30 – 0.60
Total carbon (%)	3.70	6.00	3.10	2.80	NA
Olsen phosphorus (me/100 g)	5.00	3.00	4.00	3.00	20 – 30
Sodium (me/100 g)	0.06	0.06	0.10	<0.05	0.20 – 0.50
Magnesium (me/100 g)	0.48	0.31	0.34	0.16	1.00 – 1.60
Calcium (me/100 g)	2.70	1.60	1.50	0.70	4.0 – 10.0
Potassium (me/100 g)	0.18	0.24	0.22	0.13	0.40 – 0.60
Organic matter (%)	6.30	10.4	5.30	4.80	7.0 – 17.0
pH	5.70	5.70	5.50	5.80	5.8 – 6.2

NA = not applicable.

The ambient daytime temperature differed significantly between the four study sites (Kruskal-Wallis; $X^2 = 58.275$, $df = 3$, $p < 0.001$), with site 2 having a significantly lower temperature compared to the other three sites (Figure 4a).

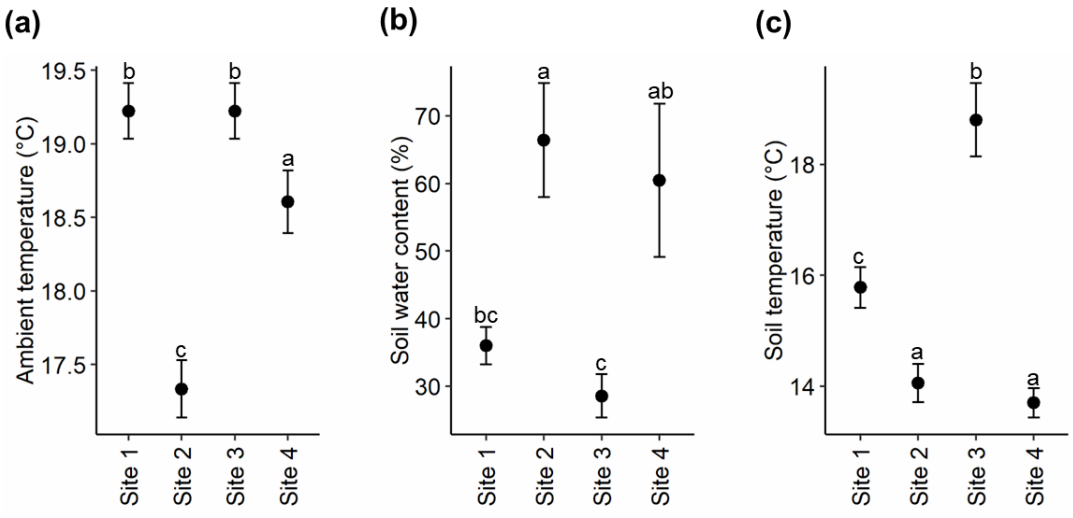

Figure 4. Comparison of (**a**) ambient daytime temperature, (**b**) soil water content and (**c**) soil temperature between sites. The y-axis showing mean ± SE values and x-axis representing the four study sites. Different letters indicate significant differences.

The soil water content (SWC) also differed significantly between the four study sites (ANOVA; $F_{3,16} = 6.206$, $p = 0.005$, Figure 4b). Site 2 had a higher SWC than site 3 (Tukey's HSD; $p = 0.011$) and site 1 (Tukey's HSD; $p = 0.046$).

There was also a significant difference in soil temperature between the four sites (Kruskal–Wallis; $X^2 = 15.736$, $df = 3$, $p = 0.001$) with site 3 having the highest soil temperature, while the lowest soil temperature was recorded at sites 2 and 4 (Figure 4c).

3.3.3 Arthropod Community Composition

Using a beating tray, arthropods were collected from the sampled heather plants (five per site) with Hemiptera, thrips, spiders and mites being the most dominant groups (Figure 5). The number of Hemiptera was significantly different between the four sites (Kruskal–Wallis; $X^2 = 15.697$, $df = 3$, $p =$

0.001). There was a greater number of Hemiptera on heather at site 4 in comparison to the other sites (Figure 5). Similarly, the number of mites on heather also differed significantly between the four sites (Kruskal–Wallis; X^2 = 13.012, df = 3, p = 0.005), with a greater number of mites at site 4. The number of thrips found on heather at site 1 was higher than the other sites, but only marginally significant (Kruskal–Wallis; X^2 = 7.698, df = 3, p = 0.053, Figure 5).

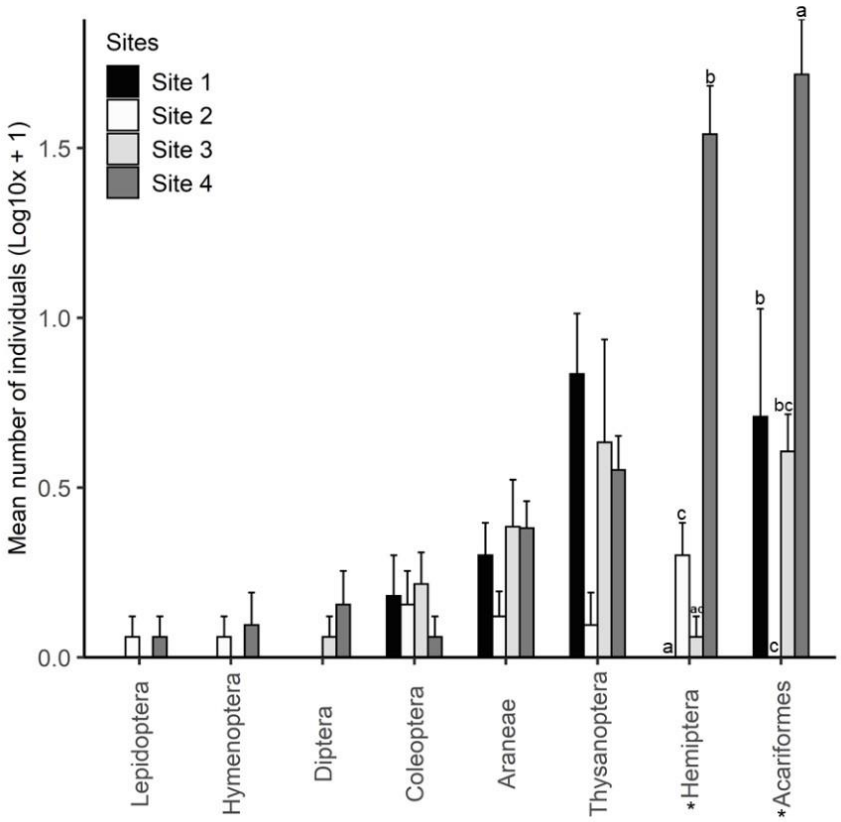

Figure 5. Arthropod community composition from study sites determined by beating a branch of target heather plants on a tray (n = 5). Bars show mean number ± SE of individuals in the respective arthropod orders. Arthropod groups with asterisks (*) were significantly different between sites.

3.3.4 Herbivore Damage on Heather

Herbivore damage on heather was recorded by counting the number of visible damage marks on the branches used for volatile collection. The distribution of herbivore damage on heather was not significantly different between the four sites (Kruskal–Wallis; $X^2 = 2.475$, $df = 3$, $p = 0.480$) although slightly higher damage was recorded on the plants at site 2 (Figure 6b).

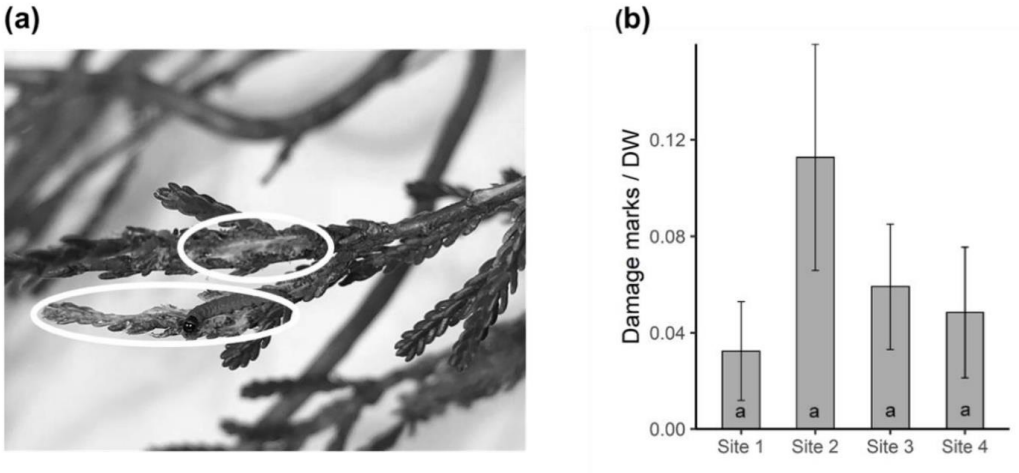

Figure 6. (**a**) Visible herbivore damage on heather. Sections in white circles were counted as two separate damage events. (**b**) Mean number ± SE of damage marks counted on target plants (n = 5) for each site expressed as damage per gram dry weight.

3.3.5 Effect of Biotic and Abiotic Factors on Volatile Emissions

We used Generalised Linear Models (GLM) to investigate the effect of biotic and abiotic factors on VOC emissions. For this purpose, the 21 volatile compounds with higher contributions to the first six components selected through the PCA were used (Appendix 1.4). The GLMs showed a significant effect of environmental variables on emissions of 14 volatile compounds, mostly fatty acid derivatives and terpenoids. However, the emissions of some compounds

((*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, (*Z*)-β-ocimene, (*E*)-β-caryophellene, γ-elemene, copaene and humulene) were not significantly affected by the factors tested in this study (Appendix 1.5).

Using K as a proxy for other nutrients, we found soil nutrients to be the main factor contributing to the differences in VOC emission of heather between sites. They significantly affected the emissions of the fatty acid derivatives (*Z*)-2-hexenol (X^2 = 51.00, *df* = 1, $p < 0.001$), (*Z*)-3-hexenyl 2-methylbutyrate (X^2 = 4.93, *df* = 1, $p = 0.026$), (*Z*)-3-hexenyl benzoate (X^2 = 50.10, *df* = 1, $p < 0.001$), (*Z*)-3-hexenyl butyrate (X^2 = 5.45, *df* = 1, $p = 0.020$), (*Z*)-3-hexenyl hexanoate (X^2 = 10.20, *df* = 1, $p = 0.001$), (*Z*)-3-hexenyl isobutyrate (X^2 = 7.23, *df* = 1, $p = 0.007$) and (*Z*)-3-hexenyl valerate (X^2 = 8.06, *df* = 1, $p = 0.005$). In addition, the emissions of terpenoids (*E*,*E*)-α-farnesene (X^2 = 15.10, *df* = 1, $p < 0.001$), α-gurjunene (X^2 = 10.10, *df* = 1, $p = 0.002$), germacrene B (X^2 = 5.79, *df* = 1, $p = 0.016$), germacrene D (X^2 = 6.08, *df* = 1, $p = 0.014$), (*E*)-β-farnesene (X^2 = 8.37, *df* = 1, $p = 0.004$) and (*E*)-DMNT (X^2 = 39.30, *df* = 1, $p < 0.001$) were significantly affected by soil nutrients (Appendix 1.5).

Temperature was the second most important factor that explained the differences in the VOC emissions of this species. Among the compounds selected through PCA, temperature significantly affected (*Z*)-2-hexenol (GLM; X^2 = 7.97, *df* = 1, $p = 0.005$), (*Z*)-3-hexenyl benzoate (GLM; X^2 = 10.90, *df* = 1, $p < 0.001$), germacrene D (GLM; X^2 = 21.10, *df* = 1, $p < 0.001$) and (*E*)-DMNT (GLM; X^2 = 5.44, *df* = 1, $p = 0.020$). The effect of herbivory on VOC emissions by heather was minimal, only significant for hexyl acetate (GLM;

X^2 = 12.50, *df* = 1, $p < 0.001$) and germacrene B (GLM; X^2 = 4.73, *df* = 1, $p <$ 0.001), while soil water content had no significant impact on the volatile emissions of heather (Appendix 1.5).

3.4 Discussion

In this study, we show that total volatile emissions by heather are highly variable ranging from 2.61 ng $gDW^{-1}h^{-1}$ to 110.985 ng $gDW^{-1}h^{-1}$ for the lowest and highest emitting plant in its invaded range in New Zealand even within the same season (summer). This species emits large amounts of terpenoids (Appendix 1.3), which is consistent with a previous report in a temperate heath ecosystem in its native range [27]. Low levels of soil nutrients appear to be particularly important in regulating the VOC emissions of heather in this habitat. The soils with lowest levels of most nutrients (site 4) in particular, potassium (K) had significantly lower emissions of most fatty acid derivatives, some sesquiterpenes and the homoterpene (*E*)-DMNT (Appendix 1.3).

The effect of soil nutrients on VOC emission by plants is still poorly documented, but previous reports suggest that nutrient depletion has a negative impact on plant volatile emissions [46-48]. The role of K has been less explored than that of other nutrients (N and P), but this macronutrient plays a key role in stomatal conductance, enzyme activity, and plant responses to a wide range of biotic and abiotic stress [49], which may all impact VOC emissions. In addition, some evidence suggests that high levels of soil K can affect the production of secondary metabolites in plants causing increased production of both non-volatile (such as phenolics, flavonoids and ascorbic acid) and volatile

compounds [50, 51].

The production of constitutive defence compounds and the inducibility of such compounds can also be controlled by nutrient availability, with some plants investing more in their production at the expense of growth under nutrient limited conditions [52]. This exemplifies the dilemma faced by plants on whether to grow or defend in response to nutrient availability, which could be vital in determining the outcome of interactions between competing plants. Further studies are required to elucidate the role of individual nutrients, as we only used potassium as a proxy for other nutrients.

The main compounds with reduced emissions at site 4, which had nutrient-poor soil, were (*Z*)-3-hexenyl acetate, (*E*)-β-caryophellene and γ-elemene (Appendix 1.3). Fatty acid derivatives including (*Z*)-3-hexenyl acetate are typically involved in direct and indirect defence by repelling herbivores and attracting their natural enemies [53-55]. Terpenoids, on the other hand, represent the largest and most diverse class of plant secondary metabolites including VOCs [56]. A single plant organ can produce multiple terpenes, which makes it difficult to assign specific roles to individual compounds in this chemical class [57]. Despite that, available literature suggests that ecological roles of terpenes include direct and indirect defence against herbivores and pathogens, attraction of pollinators and protection against abiotic stress [11, 58, 59].

Herbivory is a well-known factor affecting VOC emissions, with green leaf volatiles (fatty acid derivatives) comprising about 50% of the VOCs released by plants attacked by chewing herbivores [11, 13, 60]. In our study, herbivory had a strong positive effect on the emission of hexyl acetate, a green leaf volatile often released by damaged plants [61, 62], but negatively affected the release of germacrene B. The minimal effect of herbivore damage on VOC emissions in this study could be due to low herbivory on heather by native insects in New Zealand, and because of the lack of specialist herbivores at the selected sites. Further studies should explore the effect of the introduced biocontrol agent *Lochmaea suturalis* on the VOC emissions by heather.

Feeding damage observed on heather was caused by generalist species, and the slightly lower damage at the heather dominant site (site 1) and site 4, where another invasive plant (Scotch broom) is present, suggests that herbivorous arthropod communities are depauperate at sites where invasive plants are dominant. A recent review found that invasive plant species are often associated with an overall reduction in arthropod abundance and taxonomic richness [63]. However, changes in vegetation structure and high availability of litter and decaying vegetation caused by invasive plants can increase predators, detritivores and fungivores [63]. This agrees with the high number of spiders, oribatid mites, and fungus beetles (Cryptophagidae) found in this study (Figure 5). Most of the Hemiptera found on heather at site 4 were broom psyllids which were introduced into New Zealand in 1993 as a biocontrol agent for broom [64].

Elevated temperature is also known to increase the emission of various volatile compounds ranging from fatty acid derivatives to terpenoids [65-67], and these temperature-dependent emissions can be on both *de novo* synthesised and stored volatiles [41]. In this study, the emissions of (*Z*)-3- hexenyl benzoate, germacrene D and (*E*)-DMNT by heather were affected by temperature differences between sites. Temperature did not account for the emissions of other volatile compounds, which is likely due to the homogeneous weather during the VOC collection periods, indicating that there must be some stability in VOC emission that can withstand certain levels of environmental variation [68].

The effect of water stress on VOC emissions is not consistent in the literature and has been proposed to be dependent on plant species, duration and severity of water stress, as seen in isoprene-emitting species [41, 69]. In our study, the differences in soil water content between sites did not have a significant effect on the variability of the identified VOCs. Although there were differences between some of our study sites, it is clear that heather is a highly adaptable species, growing in conditions ranging from well-drained soils to bogs [70, 71], and is therefore not likely to be sensitive to minor fluctuations in soil water content.

A recent study found significant variation in the VOC emission of heather in response to experimentally induced elevated CO_2, drought and night-time warming over six years [27]. The results show decreased monoterpene emissions up to 40% in response to elevated CO_2. Experimentally induced drought also had a negative impact on monoterpene emissions immediately

after treatment application and in the late growing season, while experimental night-time warming increased total emissions, showing the potential impact of climate change on heather VOC emissions [27]. In contrast, monoterpenes did not appear to be particularly affected by the biotic and abiotic variables measured in our study; this suggests that plants respond differently to natural variation in their environment than to severe or long-term stressors.

3.5 Conclusions

This work explores the chemical behaviour of the highly invasive environmental weed *Calluna vulgaris* at four different sites on the Central North Plateau of New Zealand. Our study provides the first evidence suggesting that volatile emissions of *C. vulgaris* are influenced by different environmental factors, with soil nutrients (K) being a major contributor to the variation in emissions under natural conditions in its invasive range.

As this study was conducted under natural conditions, we acknowledge the possibility that variability in VOC emissions could also be linked to other variables not identified in our study. We found a common fungal volatile (1-octen-3-ol) in all sites, suggesting a possible effect of interaction of the target plants with microorganisms [45]. Furthermore, previous studies suggest that the composition of plant communities can have a strong effect on VOC emissions [72-74]. In this study, the site with lower volatile emissions had a strong presence of another invasive species, Scotch broom (*Cytisus scoparius*), whereas the site with highest emissions was dominated by heather. The other two sites had a combination of heather with native species (i.e., either mānuka

or *Dracophyllum*) evidencing differences in plant community composition (Appendix 1.1).

We therefore encourage further research to investigate the impact of plant-microbe interactions and other variables, such as neighbouring plant identity on heather volatile emissions. We also recommend exploring how these changes in a plant's emissions influence the foraging behaviour of pollinators, soil arthropods, key herbivores and their natural enemies, as well as their impact on native plants. Such studies could provide valuable information on how volatiles contribute to the successful invasion of plants into novel environments.

3.6 Acknowledgments

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3.8 Appendix 1: Supplementary information

Appendix 1.1

Table A1. Geographical coordinates for study sites.

Site	Dominant plants	Coordinates
Site 1	Heather	S39° 24.928' E175° 41.328'
Site 2	Heather and Mānuka	S39° 18.881' E175° 44.059'
Site 3	Heather – *Dracophyllum*	S39° 25.236' E175° 41.271'
Site 4	Heather – Broom	S39° 14.642' E175° 23.442'

Appendix 1.2

Table A2. Correlation test between predictor variables prior to performing GLM.

	Herbivory	SWC	Temperature	Nitrogen	Phosphorus	Potassium
Herbivory	1.0	0.2	-0.3	0.3	-0.2	0.3
SWC	0.2	1.0	-0.6	0.7	-0.6	-0.1
Temperature	-0.3	-0.6	1.0	-0.9	0.7	-0.4
Nitrogen	0.3	0.7	-0.9	1.0	-0.8	0.0
Phosphorus	-0.2	-0.6	0.7	-0.8	1.0	0.0
Potassium	0.3	-0.1	-0.4	0.0	0.0	1.0

Appendix 1.3

Table A3. List of VOCs identified from the headspace of heather. Table showing means and standard deviation rate of emission based on square root transformed data.

	Emission rate (mean ± SD ng $gDW^{-1}h^{-1}$**)**			
Compound	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Fatty acid derivatives				
Hexyl acetate	0.37 ± 0.32	0.18 ± 0.22	0.12 ± 0.16	0.10 ± 0.13
1-hexanol	0.16 ± 0.10	0.08 ± 0.12	0.12 ± 0.11	0.03 ± 0.08
(*Z*)-2-hexenol	0.67 ± 0.41	0.47 ± 0.30	0.26 ± 0.29	ND
(*Z*)-3-hexenol	0.84 ± 0.64	1.20 ± 0.40	0.74 ± 0.55	0.86 ± 0.42
(*Z*)-3-hexenyl 2-methylbutyrate	1.00 ± 0.37	0.78 ± 0.37	0.50 ± 0.39	0.32 ± 0.15
(*Z*)-3-hexenyl acetate	4.80 ± 2.52	4.66 ± 1.74	3.84 ± 2.50	2.71 ± 1.13
(*Z*)-3-hexenyl benzoate	0.49 ± 0.12	0.29 ± 0.17	0.17 ± 0.12	ND
(*Z*)-3-hexenyl butyrate	2.56 ± 0.87	2.08 ± 0.83	1.27 ± 0.78	0.81 ± 0.43
(*Z*)-3-hexenyl hexanoate	0.21 ± 0.20	0.19 ± 0.19	0.05 ± 0.12	ND
(*Z*)-3-hexenyl isobutyrate	0.47 ± 0.13	0.36 ± 0.26	0.19 ± 0.25	0.08 ± 0.12
(*Z*)-3-hexenyl valerate	0.62 ± 0.13	0.51 ± 0.27	0.30 ± 0.11	0.10 ± 0.14
Monoterpenoids				
α-pinene	0.13 ± 0.17	0.22 ± 0.31	0.06 ± 0.09	0.25 ±
β-pinene	ND	0.29 ± 0.40	ND	0.18 ± 0.17
Linalool	0.14 ± 0.32	ND	0.20 ± 0.27	0.28 ± 0.29
α-terpineol	0.10 ± 0.22	ND	0.23 ± 0.21	0.11 ± 0.25
β-myrcene	0.18 ± 0.18	0.16 ± 0.23	0.20 ± 0.19	0.09 ± 0.19
Limonene	0.23 ± 0.15	0.19 ± 0.19	0.16 ± 0.15	0.07 ± 0.11
(*Z*)-β-ocimene	0.26 ± 0.25	0.60 ± 0.38	0.40 ± 0.25	0.26 ± 0.16
Sesquiterpenes				
(*E*,*E*)-α-farnesene	1.10 ± 0.52	1.07 ± 0.32	0.96 ± 0.52	0.24 ± 0.18
α-gurjunene	0.21 ± 0.15	0.13 ± 0.13	0.15 ± 0.09	ND
(*E*)-β-caryophellene	0.51 ± 0.18	0.81 ± 0.51	0.32 ± 0.11	0.26 ± 0.17

γ-elemene	0.28 ± 0.18	0.27 ± 0.08	0.17 ± 0.12	0.10 ± 0.14
δ-cadinene	0.28 ± 0.18	0.21 ± 0.15	0.13 ± 0.09	0.12 ± 0.17
Copaene	0.07 ± 0.15	0.09 ± 0.19	ND	0.16 ± 0.16
Germacrene B	0.12 ± 0.12	0.05 ± 0.11	0.08 ± 0.11	ND
Germacrene D	0.72 ± 0.49	ND	0.09 ± 0.14	0.38 ± 0.38
(*E*)-β-famesene	0.18 ± 0.16	0.10 ± 0.14	0.13 ± 0.09	ND
Humulene	0.10 ± 0.10	0.19 ± 0.16	0.02 ± 0.05	0.03 ± 0.08
(*Z*,*E*)-α-farnesene	0.28 ± 0.63	0.65 ± 0.43	0.57 ± 0.52	ND
Homoterpenes				
(*E*)-DMNT	0.56 ± 0.35	0.33 ± 0.31	0.24 ± 0.20	ND
Other				
1-octen-3-ol	0.80 ± 0.26	0.70 ± 0.33	0.50 ± 0.08	0.42 ± 0.57
Aldehydes				
Decanal	0.33 ± 0.08	0.29 ± 0.07	0.27 ± 0.08	0.24 ± 0.10
Nonanal	0.31 ± 0.16	0.22 ± 0.15	0.22 ± 0.24	0.14 ± 0.21

*ND = not detected

Appendix 1.4

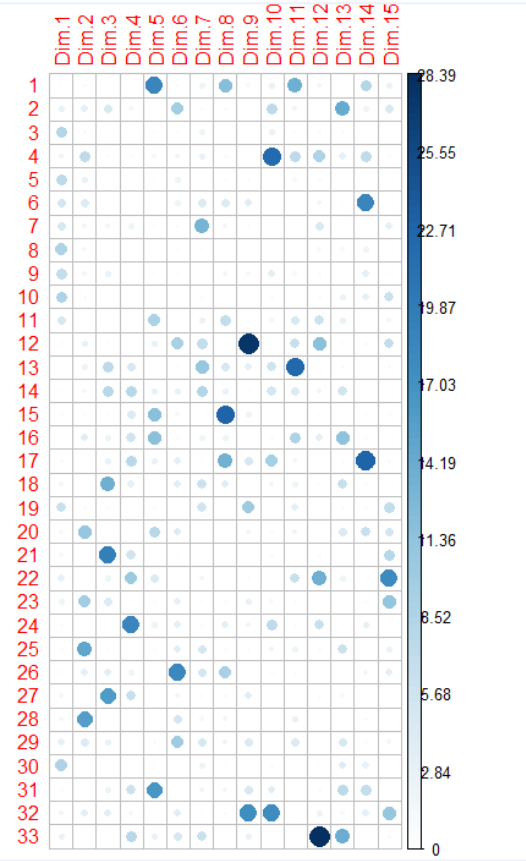

Figure A1. Contribution of volatiles compounds in various principal components. Corrplot shows 15 components (Dim1 – Dim 15). The numbers in the graph indicate the following compounds; **(1)** Hexyl acetate, (2) 1-hexanol, **(3)** (*Z*)-2-hexenol, **(4)** (*Z*)-3-hexenol, **(5)** (*Z*)-3-hexenyl 2-methylbutyrate, **(6)** (*Z*)-3-hexenyl acetate, **(7)** (*Z*)-3-hexenyl benzoate, **(8)** (*Z*)-3-hexenyl butyrate, **(9)** (*Z*)-3-hexenyl hexanoate, **(10)** (*Z*)-3-hexenyl isobutyrate, **(11)** (*Z*)-3-hexenyl valerate, (12) α-pinene, (13) α-terpineol, (14) β-myrcene, (15) β-pinene, (16) Limonene, (17) Linalool, **(18)** (*Z*)-β-ocimene, **(19)** (*E*,*E*)-α-farnesene, **(20)** α-gurjunene, **(21)** (*E*)-β-caryophellene, (22) δ-cadinene, **(23)** γ-elemene, **(24)** Copaene, **(25)** Germacrene B, **(26)** Germacrene D, **(27)** Humulene, **(28)** (*E*)-β-farnesene, (29) (*Z*,*E*)-α-farnesene, **(30)** (*E*)-DMNT, (31) 1-octen-3-ol, (32) Decanal, (33) Nonanal. Numbers in bold font indicate compounds used in GLMs.

Appendix 1.5

Table A4. Summary of GLM (gamma distribution with log-link) based on VOCs with higher contribution in PC1 – PC6. Prior to modelling, a small constant 0.001 was added to all responses and the significance of predictor variables calculated using Wald test. Bold fonts with asterisks (*) indicate significant effect of predictors on response variables.

Response	Predictor	β	CI		DF	X^2	*P*
			2.5%	97.5%			
Hexyl acetate	**Herbivory**	**1.232**	**0.549**	**1.914**	**1**	**12.500**	< **0.001***
	Temperature	0.449	-0.568	1.466	1	0.749	0.387
	SWC	-0.488	-1.399	0.423	1	1.100	0.293
	Nutrients (K)	0.028	-0.745	0.800	1	0.005	0.944
(*Z*)-2-hexenol	Herbivory	-0.136	-0.823	0.551	1	0.150	0.699
	Temperature	**1.474**	**0.451**	**2.498**	1	**7.970**	**0.005***
	SWC	-0.195	-1.111	0.722	1	0.174	0.677
	Nutrients (K)	**2.833**	**2.055**	**3.610**	**1**	**51.000**	< **0.001***
(Z)-3-hexenol	Herbivory	0.041	-0.579	0.661	1	0.017	0.896
	Temperature	0.218	-0.706	1.142	1	0.214	0.644
	SWC	0.419	-0.408	1.246	1	0.987	0.320
	Nutrients (K)	0.228	-0.473	0.930	1	0.407	0.524
(**Z**)-3-hexenyl 2-	Herbivory	-0.042	-0.644	0.561	1	0.019	0.892
methylbutyrate	Temperature	0.495	-0.403	1.393	1	1.170	0.280
	SWC	0.148	-0.656	0.952	1	0.131	0.717
	Nutrients (K)	**0.773**	**0.091**	**1.455**	**1**	**4.930**	**0.026***
(Z)-3-hexenyl acetate	Herbivory	0.287	-0.232	0.807	1	1.170	0.279
	Temperature	0.211	-0.563	0.985	1	0.284	0.594
	SWC	-0.047	-0.740	0.646	1	0.018	0.894
	Nutrients (K)	0.336	-0.252	0.923	1	1.250	0.263
(*Z*)-3-hexenyl benzoate	Herbivory	-0.134	-0.729	0.461	1	0.196	0.658
	Temperature	**1.494**	**0.607**	**2.380**	**1**	**10.900**	< **0.001***
	SWC	0.143	-0.650	0.937	1	0.125	0.723
	Nutrients (K)	**2.434**	**1.760**	**3.107**	**1**	**50.100**	**< 0.001***
(*Z*)-3-hexenyl butyrate	Herbivory	-0.151	-0.726	0.424	1	0.266	0.606
	Temperature	0.418	-0.439	1.274	1	0.914	0.339
	SWC	0.149	-0.618	0.915	1	0.144	0.704
	Nutrients (K)	**0.775**	**0.124**	**1.425**	**1**	**5.450**	**0.020***
(*Z*)-3-hexenyl hexanoate	Herbivory	-0.718	-1.518	0.082	1	3.100	0.078
	Temperature	0.081	-1.111	1.273	1	0.018	0.894
	SWC	-0.441	-1.508	0.626	1	0.656	0.418
	Nutrients (K)	**1.479**	**0.573**	**2.384**	**1**	**10.200**	**0.001***
(*Z*)-3-hexenyl	Herbivory	-0.473	-1.098	0.151	1	2.200	0.138
isobutyrate	Temperature	0.256	-0.675	1.187	1	0.290	0.590
	SWC	-0.041	-0.874	0.793	1	0.009	0.924
	Nutrients (K)	**0.970**	**0.263**	**1.677**	1	**7.230**	**0.007***
(*Z*)-3-hexenyl valerate	Herbivory	-0.102	-0.681	0.477	1	0.119	0.730
	Temperature	0.349	-0.514	1.212	1	0.628	0.428
	SWC	-0.034	-0.806	0.739	1	0.007	0.932
	Nutrients (K)	**0.950**	**0.294**	**1.605**	**1**	**8.060**	**0.005***
(Z)-β-ocimene	Herbivory	-0.074	-0.627	0.478	1	0.070	0.792

	Temperature	-0.153	-0.977	0.670	1	0.133	0.716
	SWC	0.214	-0.523	0.952	1	0.325	0.569
	Nutrients (K)	0.588	-0.037	1.214	1	3.400	0.065
(*E*,*E*)-α-farnesene	Herbivory	-0.243	-0.773	0.287	1	0.809	0.368
	Temperature	0.393	-0.396	1.182	1	0.951	0.329
	SWC	-0.085	-0.792	0.621	1	0.056	0.813
	Nutrients (K)	**1.187**	**0.588**	**1.786**	**1**	**15.100**	**< 0.001***
α-gurjunene	Herbivory	-0.156	-0.839	0.527	1	0.200	0.654
	Temperature	0.410	-0.607	1.428	1	0.625	0.429
	SWC	-0.695	-1.606	0.215	1	2.240	0.135
	Nutrients (K)	**1.252**	**0.479**	**2.025**	**1**	**10.100**	**0.002***
(*E*)-β-caryophellene	Herbivory	0.196	-0.319	0.711	1	0.556	0.456
	Temperature	-0.475	-1.242	0.292	1	1.470	0.225
	SWC	-0.203	-0.890	0.484	1	0.336	0.562
	Nutrients (K)	0.291	-0.292	0.873	1	0.955	0.329
γ-elemene	Herbivory	0.098	-0.435	0.631	1	0.130	0.718
	Temperature	0.201	-0.593	0.995	1	0.247	0.619
	SWC	0.047	-0.663	0.758	1	0.017	0.896
	Nutrients (K)	0.388	-0.215	0.991	1	1.590	0.207
Copaene	Herbivory	1.564	-0.254	3.383	1	2.840	0.092
	Temperature	0.487	-2.223	3.197	1	0.124	0.725
	SWC	0.200	-2.226	2.626	1	0.026	0.872
	Nutrients (K)	-1.111	-3.169	0.948	1	1.120	0.290
Germacrene B	**Herbivory**	**-0.803**	**-1.526**	**-0.080**	**1**	**4.730**	**0.030***
	Temperature	0.419	-0.659	1.497	1	0.581	0.446
	SWC	-0.259	-1.223	0.706	1	0.276	0.599
	Nutrients (K)	**1.005**	**0.187**	**1.824**	**1**	**5.790**	**0.016***
Germacrene D	Herbivory	0.061	-0.589	0.710	1	0.034	0.855
	Temperature	**2.267**	**1.299**	**3.235**	**1**	**21.100**	**< 0.001***
	SWC	0.493	-0.373	1.360	1	1.240	0.265
	Nutrients (K)	**-0.925**	**-1.660**	**-0.190**	**1**	**6.080**	**0.014***
Humulene	Herbivory	-0.330	-1.165	0.505	1	0.599	0.439
	Temperature	-0.448	-1.693	0.797	1	0.498	0.480
	SWC	0.411	-0.704	1.525	1	0.522	0.470
	Nutrients (K)	0.557	-0.388	1.503	1	1.330	0.248
(*E*)-β-farnesene	Herbivory	-0.550	-1.298	0.199	1	2.070	0.150
	Temperature	0.436	-0.679	1.551	1	0.587	0.443
	SWC	-0.469	-1.468	0.529	1	0.850	0.357
	Nutrients (K)	**1.250**	**0.403**	**2.097**	**1**	**8.370**	**0.004***
(*E*)-DMNT	Herbivory	-0.468	-1.178	0.242	1	1.670	0.196
	Temperature	**1.259**	**0.201**	**2.317**	**1**	**5.440**	**0.020***
	SWC	-0.216	-1.164	0.731	1	0.201	0.654
	Nutrients (K)	**2.570**	**1.766**	**3.374**	**1**	**39.300**	**< 0.001***

* Estimated coefficient (β)
* Confidence interval (CI)
* Degree of freedom (DF)
* Potassium (K)
* Soil water content (SWC)

Chapter 4

Herbivory and attenuated UV radiation affect volatile emissions of the invasive weed *Calluna vulgaris*

Adult heather beetles on blooming heather. Photo credits: ***Evans Effah***

This chapter investigates the volatile emissions of the invasive *Calluna vulgaris* in response to abiotic (UV radiation) and biotic (herbivory) environmental stressors. The chapter also explores the abundance of other arthropod groups in response to the presence of the heather beetle, and discusses results in context plant invasion. The chapter is presented in the style of the journal *Molecules*. This chapter was published by the journal as:

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radiation affect volatile emissions of the invasive weed *Calluna vulgaris*. *Molecules* 25, 3200.

Abstract

Calluna vulgaris (heather) is an aggressive invasive weed on the Central Plateau of New Zealand, where it encounters multiple factors such as comparatively high ultraviolet radiation (UV) and, herbivory by the specialist herbivore *Lochmaea suturalis* (heather beetle) that was introduced from the United Kingdom (UK) as a biocontrol agent. Like other invaders, the novel environment may not always be ideal for heather as they may need to adjust to new conditions. However, the chemical response of exotic weeds to environmental variables in their invaded range is poorly understood. Considering the vital roles of volatile organic compounds (VOCs) in plant communication, this study aimed to explore the VOC emissions of heather in response to UV exposure, and to feeding damage by *L. suturalis*. We measured VOCs produced by heather under ambient, 20% attenuated or 95% attenuated solar UV treatments, using tunnel houses clad with UV-selective filters. We also compared VOC emissions in the field at adjacent sites where *L. suturalis* was present or absent. Volatiles produced by the same target heather plants were measured at four different times in the spring and summer of 2018 – 2019, reflecting variations in beetle's abundance and phenological states of the plant. Heather plants under 95% attenuated UV produced significantly higher amounts of (*E*)-β-farnesene, decanal, benzaldehyde and benzeneacetaldehyde compared to 20% attenuated and ambient UV radiation. We also found significant differences in volatiles produced by heather plants in *L. suturalis* infested and non-infested sites on

most sampling occasions. We also recorded a lower number of generalist herbivores on heather at sites where *L. suturalis* was present. Interactions between invasive plants, a novel environment, and the native communities they invade, are discussed.

Keywords: Biocontrol agents, Environmental factors, Heather beetle, Plant secondary metabolites, Ultraviolet radiation, Volatile organic compounds

4.1 Introduction

Climate change and the spread of species beyond their natural geographic boundaries are major ways in which humans have altered the environment, with consequences to species development, fitness and competitiveness [1]. Invasive plants have high phenotypic plasticity that enhances their competitiveness [2, 3]. Also, new environments are unlikely to contain specialist herbivores creating enemy-poor or enemy-free spaces [4]. Hence invasive plants can allocate more resources towards competition instead of defence. Chemically, some invasive plants are known to release root exudates that are phytotoxic to natives [5, 6]. However, our understanding of the chemical mechanisms behind the success of invasive plants in novel environments is still limited considering that phytochemicals mediate interactions with microbes, pollinators, herbivores and their natural enemies and plant responses to stress [7].

Plants produce volatile organic compounds (VOCs) constitutively and in response to environmental stressors [8-10]. VOCs mediate several interactions between organisms acting as a source of information (infochemical) to other organisms but can also act as bioactive compounds

having direct impacts on surrounding species (e.g., allelopathy) [11]. The emitting plant can benefit directly from VOCs by attracting pollinators, repelling herbivores and reducing competition by inhibiting the growth of nearby plants [11]. However, VOC emission can also benefit the emitting plant indirectly by modifying multitrophic interactions, e.g., attracting natural enemies of their herbivores [11, 12].

The blend of plant volatiles and proportions of individual compounds can be dependent on various biotic and abiotic factors. Herbivory is the most studied biotic factor in relation to plant volatiles, and it has been linked to increased emissions of individual compounds but also to unique blends that attract the natural enemies of herbivores [8, 12-14]. For abiotic factors, the impact of temperature, soil nutrients and drought on VOCs emission has been extensively studied, while other factors such as UV radiation have received limited attention [10, 15]. Nonetheless, it is known that UV radiation can influence feeding behaviour and fitness of herbivores [16, 17], in addition to plant growth rates and morphology [16]. There is also evidence of UV-mediated VOC emissions, although responses may vary between volatile compounds and plants species [18, 19].

The impacts of biotic and abiotic stressors on VOC emissions have been poorly studied in plant invasion scenarios, and to our knowledge, only two studies have explored the effects of environmental variables on VOC emission by invasive plants [20, 21]. Under controlled conditions, elevated CO_2 levels caused increased emissions of β-caryophyllene in the invasive weed *Mikania micrantha* [20], while poor soil fertility accounted for lower

VOCs emission by the invasive *Calluna vulgaris* in the field [21]. More of these studies are needed to enhance our understanding of the mechanisms behind plant invasion, and the potential effects of invasive plants' volatile compounds on the native flora, fauna and microbiota.

This study aimed to explore VOC emissions of the invasive *Calluna vulgaris* (heather) in its invasive range in response to abiotic (UV radiation) and biotic (herbivory) factors. Heather is a perennial shrub in the Ericaceae family that was introduced from Europe into New Zealand's North Is. Central Plateau by early European settlers in 1912 [22, 23]. At present, it is the most widespread invasive weed in that area outcompeting native plants and displacing their associated fauna.

In New Zealand, new heather shoots reach their final length between late November and early January, the first flowers open between mid-January and early February, and plants keep mature photosynthetic tissue throughout the autumn and winter months (March – August) [24]. Many authors have provided a detailed description of the topography, soils and environmental conditions associated with heather in New Zealand [23, 25, 26] and a recent study documented the natural variation in heather volatile emissions on the Central Plateau [21].

Despite heather being prolific in this ecosystem, it faces two important environmental challenges. Firstly, UV radiation levels in New Zealand are considerably higher compared to heather's native range in Europe [27]. This may induce changes to the plant's biochemistry [28]. Secondly, the heather beetle (*Lochmaea suturalis*) was introduced as a biocontrol agent into the

Tongariro National Park in 1996 and is starting to have significant impacts [29, 30].

We investigated volatile production of heather in response to these abiotic and biotic factors; i.e., exposure to UV radiation and feeding damage by *L. suturalis* in the tunnel house and under natural conditions respectively. Using screened tunnel house conditions, we measured VOCs produced by heather under ambient, 20% attenuated or 95% attenuated UV. In the field study, VOC measurements were made at four different sampling times in two adjacent sites where the heather beetle was present or absent. The sampling times reflected the plant's responses during different phenological states of heather plants and abundance of the herbivore. We also collected arthropods on heather plants, to estimate the herbivore load and how the distribution of other species might be affected by the presence of *L. suturalis.* Based on existing literature, we hypothesised that volatile emissions will be higher for plants exposed to high levels of UV radiation and beetle damage. We also expected to observe variation in the arthropod community composition between *L. suturalis* infested and non-infested sites likely due to changes in plant food quality, increased competition or induced plant defences.

4.2. Materials and Methods

4.2.1 Experimental design

4.2.1.1 UV Experiment

Heather plants of similar size were collected from a natural population at Taurewa (Long. 175.556591 – Lat. -39.081535) on the Central Plateau,

North Island, New Zealand during September 2018 and transplanted into plastic pots (23 cm × 26 cm) maintaining the root-bound soil intact.

The plants were maintained outdoors at Massey University Plant Growth Unit, Palmerston North, New Zealand (Long. 175. 614600 Lat -40.377474) for six weeks, then on 1 November transferred to a tunnel house facility and maintained under one of the three randomly allocated treatments. 40 heather plants were placed into each treatment. Treatment one used a polyethylene UV-modifying filter which achieved >95% attenuation of ambient UV ['UV-opaque'] (Lumisol '019', BPI Visqueen, UK). Treatment two achieved 20% attenuation of ambient UV ['UV-transparent'] (Lumisol '018', BPI Visqueen, UK); the UV-modifying filters are described in more detail in [31]. Although some variation in Photosynthetically Active Radiation (PAR: 400 – 700 nm) was recorded under the films at the date of sampling (Appendix 2.1), these differences were not significant and were likely due to variable cloud cover at the time of measurement, only UV-A and UV-B measurements varied significantly between the films (Appendix 2.1).

A dividing curtain of Lumisol '019' was installed at the junction of the two screen types to prevent leakage of UV between treatments. The third treatment was outside and adjacent to the tunnel house in unscreened ambient light. All the potted plants used in this experiment were in field-collected soil and were watered using individual drip irrigation for three minutes, four times daily, with no additional soil or nutrients. To maintain more equitable tunnel house temperatures, fans were installed and programmed to run at a setpoint of 20° C. Temperature and humidity were recorded continuously using HOBO data loggers (Table 1), and the plants were exposed to these

treatments for 75 days. For VOC collection, plants were transferred directly from the tunnel house into a temperature-controlled room (25° C) under continuous lighting, and volatiles collected on the same day.

Table 1. Microclimatic measurements for tunnel house and ambient treatments.

	Treatment (mean ± SD)		
Variable	**Ambient**	**20% attenuation**	**95% attenuation**
Temperature (ºC)	18.56 ± 3.96	21.98 ± 5.76	22.17 ± 5.59
Humidity (%)	74.76 ± 13.15	64.19 ± 15.49	64.73 ± 16.09

4.2.1.2 Field observation of herbivore damage on heather

Herbivore damage on heather was observed during summer (November 2018 to March 2019). Two adjacent sites, 880 m a.s.l., and of the same volcanic soil type were identified within the Waiouru Military Training Area on the Central Plateau, North Island, New Zealand (Treatment site Long. 175.687533 – Lat. -39.406130, Control site Long. 175.685303 – Lat. -39.431929). At the time of the experiment, the biocontrol agent, *Lochmaea suturalis* was present at one of the sites (treatment site) but absent from the other (control site). Volatiles from eight target plants were measured in each treatment.

At the initial setup, and during the sampling period, the number and stage of beetles at each of the sites was determined by sampling with the beating tray technique, as described by [21] on three plants adjacent to the targeted VOC sampling plant. Collected heather beetles and other invertebrate specimens were preserved in 70% ethanol and later classified to order.

To elucidate heather's response to feeding damage caused by *L. suturalis* in combination with different plant phenological stages, volatiles and invertebrates were collected on four different occasions on 14 November 2018, 11 December 2018, 31 January 2019 and 25 March 2019 during sunny and dry weather conditions.

4.2.2 VOCs sampling

For both UV-B and herbivory experiments, foliar volatiles from heather plants was collected using the "push-pull" headspace sampling technique and analysed following the same protocol as in [21]. Heather foliage was enclosed in a new oven bag, and carbon filtered air was pushed into the bag (1.70 L/min) and simultaneously pulled out (1.20 L/min) through PTFE tubes using a PVASS22 pump. VOCs in the air surrounding the enclosed foliage was collected using a volatile collection trap with 30 mg HayeSep Q adsorbent (Volatile Assay Systems Rensselaer, NY, USA). The collection traps were eluted using 200 μL of 99% hexane with 10 ng/mL nonyl acetate ($C_{11}H_{22}O_2$) (Sigma Aldrich) and analysed using gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu technologies). Operation conditions of the GC-MS and identification of compounds followed the protocol described in [21].

4.2.3 Data analysis

UV response data were square-root transformed and analysed using Principal component analysis (PCA). PCA was performed using the "FactoMiner" package [32]. Individual compounds with higher contributions to the first and second principal components were selected for subsequent analyses.

The volatile profiles of heather plants from *Lochmaea suturalis* infested and non-infested sites were compared using permutational multivariate analysis of variance (PERMANOVA) [33, 34]. PERMANOVA was ran using the "adonis" function in the vegan package R [35]. When groups were significantly different, similarity percentage (SIMPER) was used to identify compounds contributing to the differences [31]. The patterns in VOC emissions between groups were visualised using non-metric multidimensional scaling (NMDS), also with the vegan package. Both PERMANOVA and NMDS were based on Bray-Curtis dissimilarities using square root transformed VOCs data. All statistical analyses were performed using R.

Major volatile classes and the relative proportion of individual compounds selected through PCA were compared between the groups using the Kruskal-Wallis or Wilcoxon rank sum test. Arthropod abundance between sites was also compared using the Wilcoxon rank sum test. For this comparison, *L. suturalis* was excluded.

4.3 Results

4.3.1 Volatile organic compounds emissions under UV treatments

Foliar volatile compounds produced by heather plants exposed to ambient, 20% attenuated and 95% attenuated UV were collected using the "push-pull" headspace sampling technique. Compounds were grouped into major chemical classes and compared between the treatments. Except for sesquiterpenes (Kruskal-Wallis; $X^2 = 7.74$, df = 2, $P = 0.021$), the proportions of major VOC classes did not differ between the treatments, although fatty

acid derivatives (FADs) and total volatile emissions were marginally higher for plants exposed to 20% attenuated UV (Fig.1).

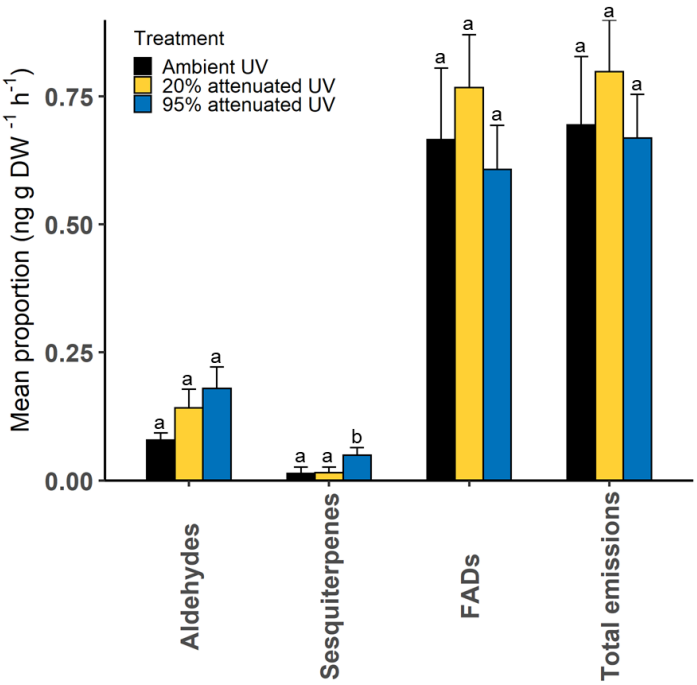

Figure 1. Proportions of major chemical classes in heather plants exposed to ambient and attenuated UV (n = 10 for each treatment). Y-axis shows (log10x +1) transformed mean ± SE proportion of compound classes. Different letters indicate significant differences.

Principal component analysis (PCA) was performed based on all the volatile compounds identified from heather plants under each UV treatment. Results of the PCA show separation between the VOCs produced by heather exposed to 95% attenuated and ambient UV. The first and second principal components (PC1 and PC2) explained 59.8% of the variation in emissions between the three treatments. PC1 was characterised by the fatty acid derivatives (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenyl butyrate. PC2 was characterised by aldehydes benzeneacetaldehyde, benzaldehyde, decanal and a sesquiterpene (*E*)-β-farnesene (Fig. 2a).

The relative proportions of compounds with higher contributions to PC1 and PC2 (Appendix 2.2) were compared between the three UV treatments (Fig. 2b). The results show significant differences in the proportion of (*E*)-β-farnesene (Kruskal-Wallis; X^2 = 7.74, df = 2, P = 0.021), benzaldehyde (Kruskal-Wallis; X^2 = 17.25, df = 2, P < 0.001), benzeneacetaldehyde (Kruskal-Wallis; X^2 = 11.96, df = 2, P = 0.003) and decanal (Kruskal-Wallis; X^2 = 23.84, df = 2, P < 0.001) with higher emissions from heather under 95% attenuated UV (Fig. 2b).

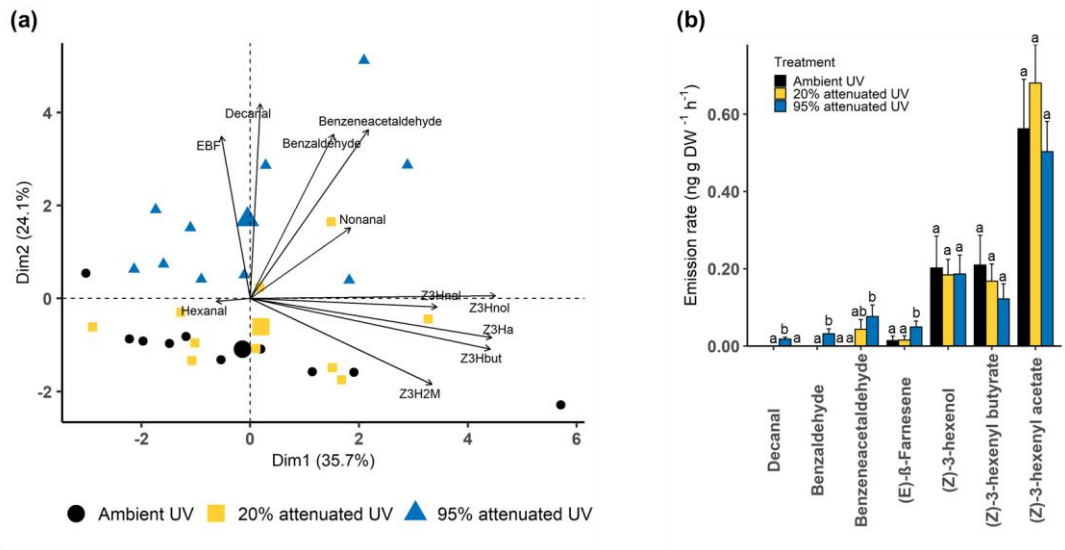

Figure 2. (a) PCA biplot based on the volatile compounds produced by heather under ambient, 20% attenuated and 95% attenuated UV. (b) the relative proportion of compounds with higher contributions to PC1 and PC2 between the three UV treatments (n = 10). Y-axis of barplot (b) shows (log10x +1).

4.3.2 Field observation of herbivore damage on heather

4.3.2.1 Plant health and Lochmaea suturalis phenology during the experiment

The phenology of *L. suturalis* and heather plants were visually inspected during each sampling period. On 14 November 2018, the plants at the

infested site were reasonably healthy but showing signs of previous damage and not as lush and green as the control site. During the second sampling on 11 December 2018, any new season's growth on plants at the infested site had been chewed by the beetle. They were showing signs of desiccation while control plants remained undamaged with much newer season's growth evident. On 31 January 2019, plants at the infested site remained desiccated, showed increased browning and little sign of flowering while control site plants displayed a profusion of flowers. During the fourth sampling on 25 March 2019, infested site plants were very brown and desiccated while control plants were green with matured current season growth.

Lochmaea suturalis also progressed through different developmental stages at the beetle-infested site during the sampling period (Fig. 3). From the first collection on 14 November 2018, when plants were at the leaf budding stage, adult *L. suturalis* were most abundant. On 11 December 2018, during the second sampling, the foliage was green and lush, and collections were dominated by first instar *L. suturalis*. During the third sampling on 31 January, 2019 when plants were flowering third instar larvae and adult *L. suturalis* were more abundant. During the fourth and final sampling on 25 March 2019 plant foliage had matured, and the beetle-infested site was again dominated by newly emerged adult *L. suturalis*. The control plants remained beetle-free for the entire duration of the experiment.

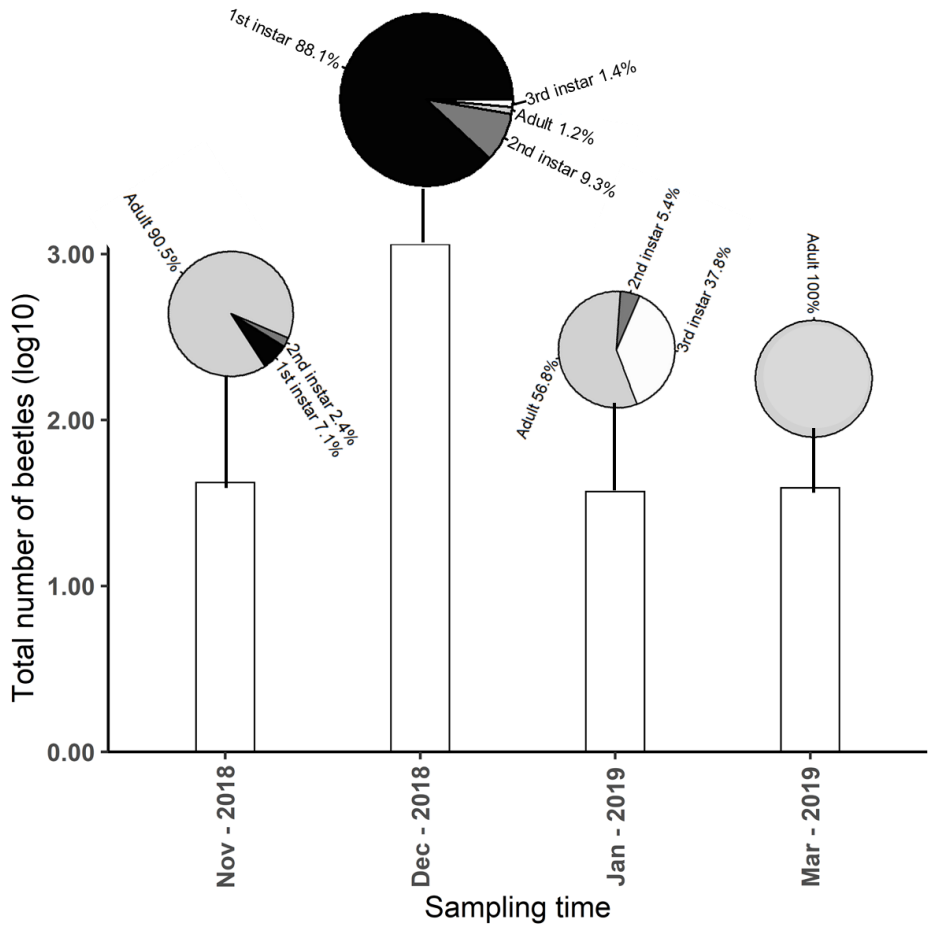

Figure 3. The abundance of *L. suturalis* during the four VOC measurements periods. Bar indicates the total number of *L. suturalis* present and pie charts show the proportion of each developmental stage at a given sampling time.

4.3.2.2 Volatile profiles of heather at Lochmaea suturalis infested and non-infested sites

First, we compared the proportion of major chemical classes produced by heather at *L. suturalis* infested and non-infested sites. Overall, the results reveal variations in some chemical classes between sites at each sampling time (Fig. 4).

Aldehydes (Wilcoxon sum rank test; $P = 0.024$) and monoterpenoids (Wilcoxon sum rank test; $P = 0.005$) were produced in significantly higher amounts at the beetle-infested (beetle present) site in November 2018, when

the site was dominated by adult *L. suturalis* and plants foliage was budding despite signs of damage (Fig. 4a).

In December 2018, when foliage at the infested site was showing signs of beetle grazing and desiccation, with first and second instar *L. suturalis* being dominant, the proportion of fatty acid derivatives (Wilcoxon sum rank test; $P = 0.005$), monoterpenoids (Wilcoxon sum rank test; $P = 0.029$), homoterpenes (Wilcoxon sum rank test; $P = 0.040$) and total VOC emissions (Wilcoxon sum rank test; $P = 0.005$) varied significantly between the sites (Fig. 4b).

Only the proportion of alcohols differed significantly between the sites (Wilcoxon sum rank test; $P = 0.043$) when plants at the non-infested site (heather absent) were heavily flowering while third instar and adult *L. suturalis* were dominant on the desiccated poorly flowering heather at the beetle present site in January 2019. The proportion of monoterpenoids was higher at the beetle-infested site, but this was not significant (Fig. 4c).

Except for sesquiterpenes (Wilcoxon sum rank test; $P = 0.032$), there were no significant differences in the major chemical classes between sites in VOCs measured in March 2019 despite foliage at the non-infested site being green and mature as compared to the brown desiccated plants at the infested site where newly emerged adult *L. suturalis* were present. (Fig. 4d).

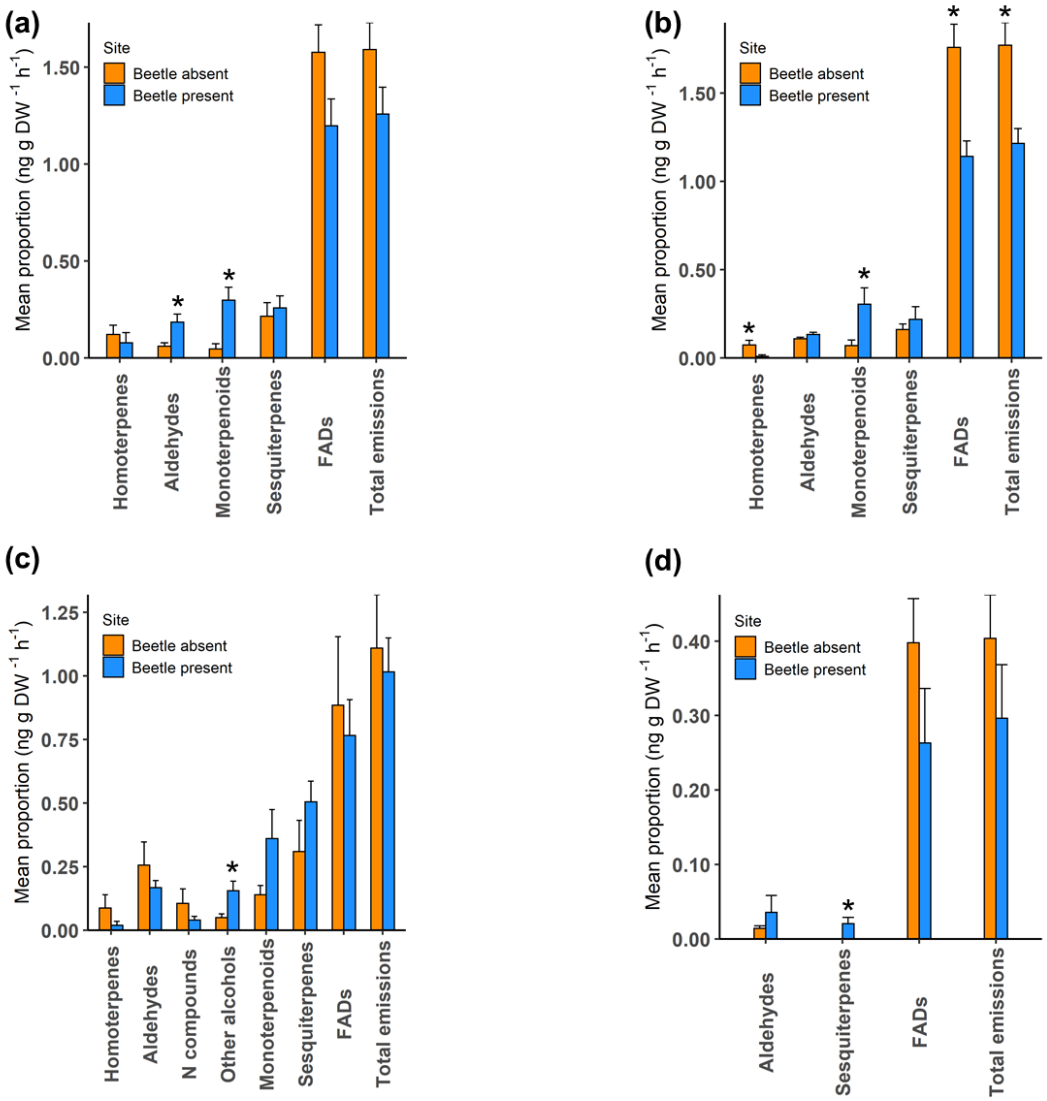

Figure 4. Comparison of major chemical classes between sites at (a) Nov 14 2018, (b) Dec 11 2018, (c) Jan 31 2019 and (d) Mar 25 2019. Y-axis shows (log10x +1) transformed mean ± SE proportion of chemical classes and asterisk indicate a significant difference. N = 7 for the beetle present site in (c), otherwise 8 replicates per treatment at each sampling time.

Permutational multivariate analysis of variance (PERMANOVA) was used to compare the overall volatile profile of heather plants at the beetle-infested and non-infested sites. We found variation in volatile profiles between heather from beetle-infested (beetle present) and non-infested (beetle absent)

sites in November 2018 (PERMANOVA; Pseudo-$F_{1,14}$ = 3.21, P = 0.028). The similarity percentage analysis revealed that the homoterpene (*E*)-DMNT, six fatty acid derivatives, three sesquiterpenes and monoterpene (*Z*)-*β*-ocimene were the main compounds contributing to the observed variation (Fig. 5, Appendix 2.3).

Heather's volatile profiles also varied between sites in December 2018 (PERMANOVA; Pseudo-$F_{1,14}$ = 6.66, P = 0.006). Fatty acid derivatives (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*Z*)-3-hexenal, (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl 2-methylbutyrate and terpenes (*Z*)-β-ocimene, germacrene D, (*E*)-β-caryophyllene were the main compounds accounting for the observed differences (Fig. 5, Appendix 2.3).

Similar segregation was observed in the composition of heather's volatile profile between sites in January 2019 (PERMANOVA; Pseudo-$F_{1,13}$ = 3.55, P = 0.022). A total of 54 volatile compounds were identified from plants at beetle-infested and non-infested sites. The similarity percentage analysis revealed that 24 compounds were the main drivers of the observed pattern in VOC emissions between sites (Fig. 5, Appendix 2.4).

Unlike the other sampling times, volatile profiles did not differ between heather at beetle-infested and non-infested sites in March 2019 (PERMANOVA; Pseudo-$F_{1,14}$ = 2.14, P = 0.112, Fig. 5)

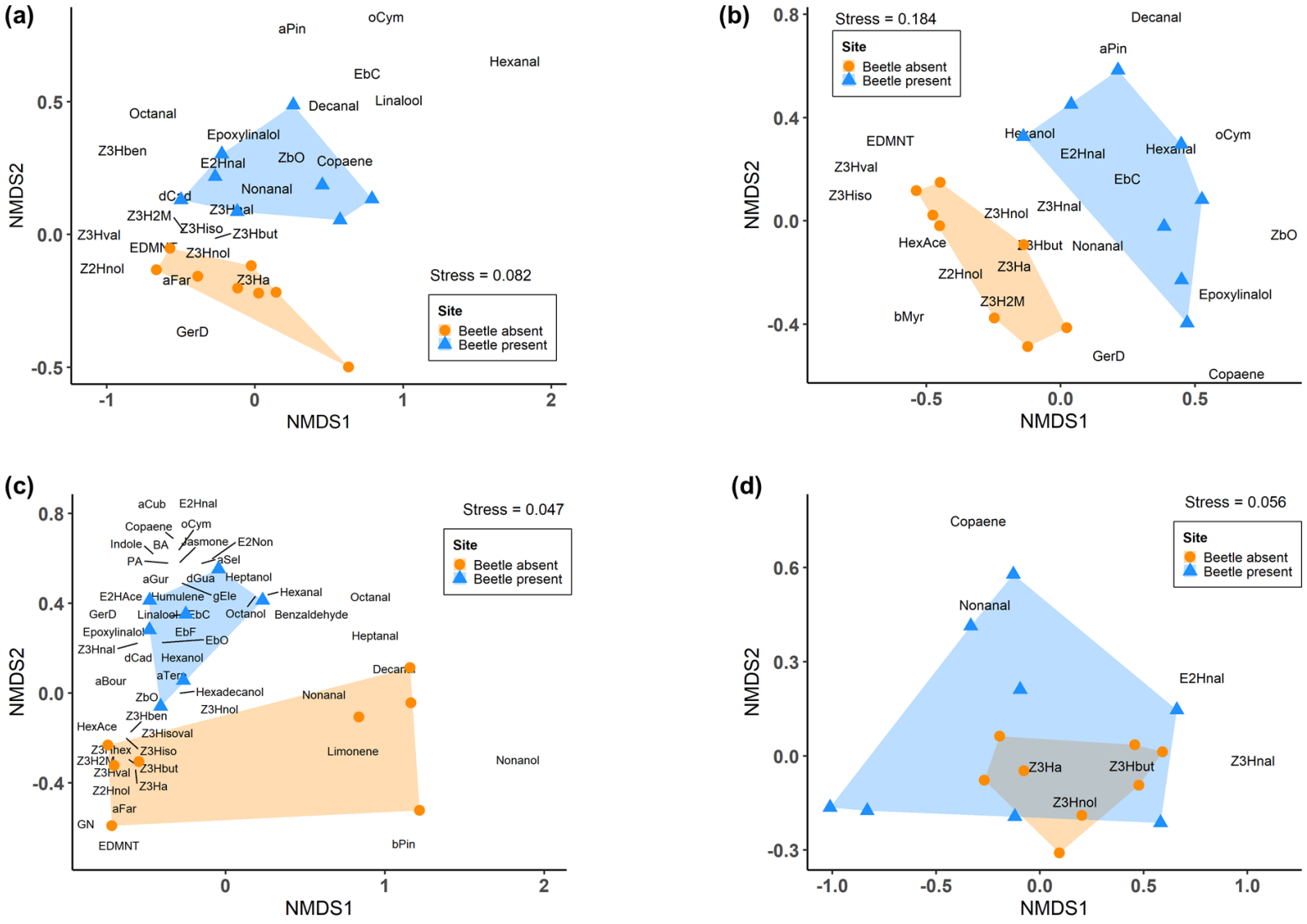
(a)
aPin
oCym
Hexanal
EbC
Decanal
Linalool
Octanal
Epoxylinalol
Z3Hben
E2Hnal
ZbO
Copaene
Nonanal
dCad
Z3H2M
Z3Hval
Z3Hiso
Z3Hbut
EDMNT
Z3Hnol
Z2Hnol
aFar
Z3Ha
GerD
Stress = 0.082
Site
Beetle absent
Beetle present
NMDS1
NMDS2
(b)
Stress = 0.184
Decanal
aPin
Hexanol
oCym
EDMNT
E2Hnal
Hexanal
Z3Hval
EbC
Z3Hiso
Z3Hnol
Z3Hnal
ZbO
HexAce
Z3Hbut
Nonanal
Z2Hnol
Z3Ha
Epoxylinalol
Z3H2M
bMyr
GerD
Copaene
(c)
Stress = 0.047
aCub
E2Hnal
Copaene
oCym
Indole
BA
Jasmone
E2Non
PA
aSel
aGur
dGua
Heptanol
E2HAce
Humulene
gEle
Hexanal
Octanal
GerD
Linalool
EbC
Octanol
Benzaldehyde
Epoxylinalol
EbF
Heptanal
Z3Hnal
EbO
dCad
Hexanol
aBour
aTer
Decanal
ZbO
Hexadecanol
Nonanal
Z3Hnol
Z3Hben
HexAce
Z3Hisoval
Z3Hhex
Z3Hiso
Limonene
Nonanol
Z3H2M
Z3Hval
Z3Hbut
Z2Hnol
Z3Ha
aFar
GN
EDMNT
bPin
(d)
Stress = 0.056
Copaene
Nonanal
E2Hnal
Z3Ha
Z3Hbut
Z3Hnal
Z3Hnol
NMDS1
NMDS2

Figure 5. NMDS plot for VOCs identified from heather at adult *L. suturalis* infested (beetle present) and non-infested (beetle absent) sites in (a) November 2018, (b) December 2018, (c) January 2019 and (d) March 2019. N = 7 for the beetle present site in (c), otherwise 8 replicates per treatment were done in all sampling times.

Abbreviations: (*Z*)-3-hexenyl benzoate (Z3Hben), δ-cadinene (dCad), (*E*)-2-hexanal (E2Hnal), (*Z*)-3-hexenyl 2-methylbutyrate (Z3H2M), (*Z*)-3-hexenyl valerate (Z3Hval), (*E*)-4,8-dimethyl-1,3,7-nonatriene (EDMNT), (*Z*)-2-hexenol (Z2Hnol), germacrene D (GerD), (*E*,*E*)-α-farnesene (aFar), (*Z*)-3-hexenyl acetate (Z3Ha), (*Z*)-3-hexenol (Z3Hnol), (*Z*)-3-hexenal (Z3Hnal), (*Z*)-3-hexenyl butyrate (Z3Hbut), (*Z*)-3-hexenyl isobutyrate (Z3Hiso), (*Z*)-β-ocimene (Zbo), α-pinene (aPin), (*E*)-β-caryophyllene (EbC), o-cymene (oCym), hexyl acetate (HexAce), β-myrcene (bMyr), (*E*)-β-farnesene (EBF), (*Z*)-3-hexenyl hexanoate (Z3Hhex), (*Z*)-3-hexenyl isovalerate (Z3Hisoval), geranyl nitrile (GN), phenylethyl alcohol (PA), α-bourbonene (aBour), α-cubebene (aCub), benzyl alcohol (BA), α-gurjunene (aGur), α-selinene (aSel), α-terpineol (aTerp), β-pinene (bPin), γ-elemene (gEle), δ-guaiene (dGua), (*E*)-β-ocimene (EbO), (*E*)-2-hexenyl acetate (E2HAce), (*E*)-2-nonenal (E2Non).

4.3.2.3 Arthropod community composition between sites

During each sampling period, arthropods present on heather plants were collected using the beating tray technique. Collected specimen were identified to order level and compared between the two sites. Overall, there were differences in the arthropod composition (excluding *Lochmaea suturalis*) on heather plants between infested and non-infested sites (Fig. 6). The results show significant differences between sites for the number of Araneae collected in November 2018 (Wilcoxon sum rank test; $P = 0.012$) and March 2019 (Wilcoxon sum rank test; $P = 0.008$) with higher numbers at the site where *L. suturalis* was not present (Fig. 6a and d). Similarly, higher numbers of Thysanoptera were recorded at the site where *L. suturalis* was not present in November 2018 (Wilcoxon sum rank test; $P = 0.003$) and January 2019 (Wilcoxon sum rank test; $P = 0.009$) (Fig 6a and c). The number of Thysanoptera was only marginally (Wilcoxon sum rank test; $P = 0.076$, Fig. 6b) higher at the site where *L. suturalis* was not present in December 2018.

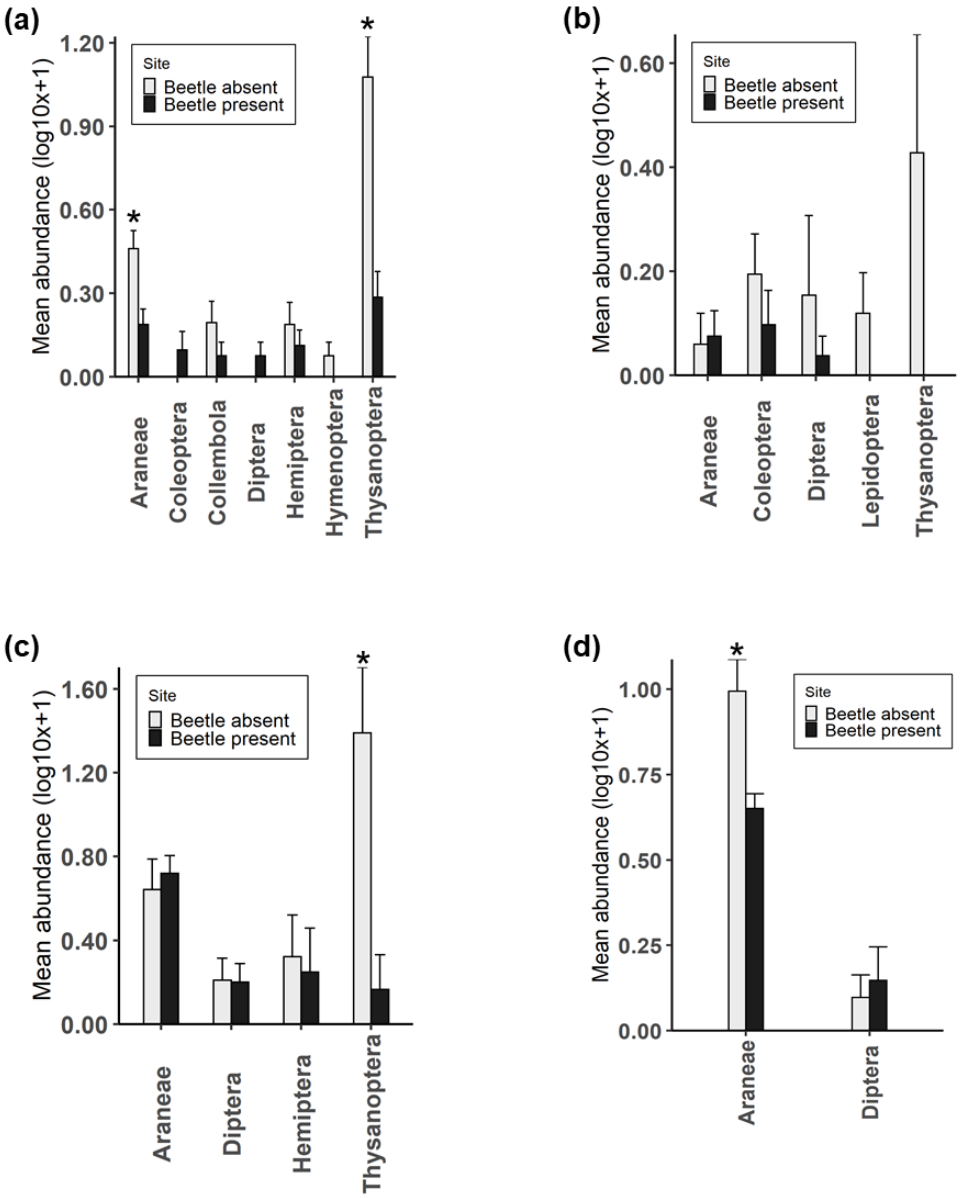

Figure 6. Comparing the abundance of arthropod other than *L. suturalis* on heather between sites. Samples collected on (a) November 2018, (b) December 2018, (c) January 2019 and (d) March 2019 using the beating tray technique (n = 3). Bars show mean ± SE and asterisk indicates a significant difference between sites.

4.4 Discussion

4.4.1. UV mediated volatile emissions

Plant responses to UV radiation differ between species, genotypes and even sex [36-38], but generally, exposure to high UV doses causes several adaptive mechanisms in plants including the production of new secondary compounds, increase in UV-absorbing compounds and antioxidant [39-41]. Some plants may increase growth and productivity in response to UV exposure [42, 43], although adverse outcomes have also been documented [44, 45]. UV radiation also induces the production of other chemicals in plants, including VOCs [37, 46]. The effect of UV exposure on heather's volatile emissions has not been previously documented, but there is evidence of a reduction in the concentration of the amino acid isoleucine, which is a VOC precursor, in response to enhanced UV-B [47].

Results from the present study demonstrate that long term exposure to ambient UV negatively affects the amount of VOC emitted by heather. Compared to ambient UV (highest UV level), exposure to 95% attenuated UV was associated with higher emissions of sesquiterpenes such as (*E*)-β-farnesene and some aldehydes such as benzaldehyde and benzeneacetaldehyde. In agreement with our results, [48] found that volatile emissions of juvenile *Eucalyptus globulus* also decreased after two days of exposure to elevated UV-B, with reductions in terpenes and aldehydes. The amount of monoterpenes including α-phellandrene, α-thujene and o-cymene decreased significantly in UV-B exposed plants, while aldehydes particularly benzaldehyde, on the other hand, showed an initial increase after stress removal but decreased after that [48]. Similar studies report a reduction in

total terpenes by *Pistacia lentiscus* in response to elevated UV addition [49] and the enhanced production of amino acid-derived volatiles by attenuated UV radiation in *Vitis vinifera* [50].

On the contrary, a number of studies have measured increased VOC emissions under elevated UV radiation [37, 51-53]. An increase in VOC emissions under elevated UV radiation, particularly UV-B, has been suggested as a plant defensive mechanism to protect tissues from adverse effects [8, 54]. The discrepancies between studies may be a result of species-specific responses to UV radiation, but the effect likely varies depending on the duration and intensity of UV exposure. For instance, emission of terpenoids by indoors-grown Norway spruce (*Picea abies*) after 4 hours of UV-B exposure showed an increased amount of bornyl acetate, borneol, myrcene, and limonene within the first three days but emissions returned to normal after three weeks of treatment [55]. Whereas needle monoterpene and sesquiterpene emission of field-grown Norway spruce seedling did not respond to continuously enhanced UV-B radiation up to 30% above the ambient level [56].

Another factor to consider may be the initial state of the plant. In our study, plants were collected from the field, where they had been exposed (and possibly adapted) to high UV conditions prior to the experiment. Hence, it is feasible that they would react differently to plants grown in a greenhouse or under low UV conditions. We, therefore, encourage future studies to investigate whether a reduction in metabolites is typical behaviour for this

species under elevated UV radiation, or if changes in VOC emissions are context-dependent.

4.4.2 Damage by specialist herbivore and VOC emissions

Herbivore damaged plants produce many volatile compounds that may differ from undamaged plants. Such damage-induced plant volatiles can be released from the damaged site, or undamaged parts of attacked plants and nearby undamaged plants [13, 57, 58]. These damaged-induced VOCs play critical roles in plant defence against enemies [8, 13]. Our results demonstrate that heather plants emit blends of volatile compounds that vary between *Lochmaea suturalis* damaged plants and those from a population where the beetle is not present. The results also suggest that heather's response to feeding damage by *L. suturalis* varies with the abundance of the beetle and the plant's phenology. To our knowledge, this is the first report of a plant's VOC response to feeding by its specialist herbivore in combination with different plant phenology and abundance of the herbivore under natural conditions in its introduced range.

Several studies indicate that lipoxygenase products, including the C_6 green leaf volatiles, are readily released from broken tissues and constitute a large percentage of the VOCs emitted by plants attacked by chewing herbivores [8, 59]. However, feeding damage by *L. suturalis* did not cause higher emission of these group of compounds in our study. Perhaps this is because plants in the field are subject to previous and continuous damage, unlike plants in most controlled laboratory studies that are naïve to herbivore damage [60]. In fact, higher emissions of (*Z*)-3-hexenyl acetate, (*Z*)-3-

hexenyl 2-methylbutyrate, (*Z*)-3-hexenol and (*Z*)-3-hexenyl butyrate were instead found at the site where *L. suturalis* was not present.

On the other hand, terpenoids, which are more expensive to produce compared to other compounds [61], were emitted in higher amounts at the *Lochmaea suturalis* infested site. Emissions of (*Z*)-β-ocimene, (*E*)-β-caryophyllene, (*E*)-β-farnesene, δ-cadinene and δ-guaiene were significantly higher at the *L. suturalis* infested site (Appendix 2.3 and Appendix 2.4), suggesting a possible selective defence by heather against generalist and specialist herbivores or production of these compounds being dependant on the level of herbivory. The beetle-free site was not herbivore-free as evidenced by the number of thrips and lepidopteran larvae caught at this site. Besides, the higher emissions of homoterpenes and fatty acid derivatives from heather at the beetle absent site may probably indicate thrips damage. Also, VOC profiles changed over time in relation to abundance and growth stages of the herbivore, suggesting that plant responses can be quite specific to the identity and developmental stage of the attacker. This supports earlier reports indicating that the composition of volatiles emitted by attacked plants can depend on the stage, abundance and feeding pattern of herbivores [62-65].

We also acknowledge that the phenology and physiology of target plants at sampling times had a significant impact on the composition of measured plant volatiles. Our results show that the differences between VOC profiles of *L. suturalis* infested and uninfested plants were more marked at the leaf budding and flowering stage and negligible past the flowering stage. This

indicates that young and reproductive tissue are better defended, as evidenced by their higher VOCs emissions than mature non-reproductive organs [66, 67]. Besides, although both beetle-infested and non-infested sites are of the same volcanic soil type, local nutrient pools might be altered at the beetle-infested likely due to decomposition of dead plants, which may influence heather's VOC emissions. This creates an avenue for future investigations.

4.4.3 Potential ecological impact of variable emissions

Changes in VOC emissions by heather in response to abiotic or biotic factors could have different effects on other members of the community, and may also directly or indirectly affect the emitting plant. Most attacked plants can reduce herbivore loads by producing volatile compounds that can directly repel herbivores or modify the interaction between herbivores and their natural enemies [8, 12, 68]. We did not see any evidence of increased predation at the *Lochmaea suturalis* infested-sites but rather a displacement of other herbivores. Specialist herbivores like *L. suturalis* may be less negatively impacted by the defences built up by their host plant compared to generalists [69], which may explain the lower abundance of other arthropods especially thrips and lepidopteran larvae at the *L. suturalis* infested site. However, the variation in arthropod community between the beetle absent or present sites may also be attributed to changes in the quality and quantity of plant food source, and herbivores avoiding heather plants being attacked by *L. suturalis* because the food is being removed.

A review exploring the effects of changing environmental factors on tritrophic interactions found that VOC emissions induced by some abiotic factors can influence the communication between plants, herbivores and the natural enemies of herbivores, but this may vary between stressors and organisms [10]. In plant invasion scenarios, changes in the amount and composition of plant VOCs in response to stresses could impact plant competition between native and invasive plants by interfering with or interrupting signals between neighbouring plants and other organisms or by disrupting communication altogether. Whereas this phenomenon has not yet been studied, this information is vital to broadening our understanding of plant invasions, particularly in the context of climate change since abiotic factors such as temperature, drought and CO_2 are expected to rise in the coming years [70].

In addition to the potential disruption of multitrophic interactions, the emitted compounds emitted under stressful conditions may directly affect nearby native plants, but to our knowledge, there is only one study exploring such effects within a plant invasion context [20]. The authors showed that elevated CO_2 levels cause increased emissions of the volatile compound β-caryophyllene in one of the worst invasive weeds in the world (*Mikania micrantha*), which was linked to enhanced phytotoxicity and allelochemical properties against various potential competitors. This is also a research area that requires further attention.

Finally, the production of plant volatiles can have varying costs on raw materials and biosynthetic enzymes, and plants may reduce such costs by

utilising individual compounds in multiple roles or catabolising compounds that are not needed [61]. There may also be a trade-off between the emission of plant volatiles and the use of those resources for growth and reproduction [71, 72], which could be important for invasive species like heather that outcompete competitors through rapid growth and high reproduction rates. Therefore, it is not surprising that some stressors may have adverse effects on VOC production and emission by invasive plants.

4.5 Conclusions

This study shows that both abiotic (UV radiation) and biotic (herbivory) factors can influence volatile emissions by an invasive species. Plant responses are context-dependent varying with the intensity and duration of exposure to abiotic stress, but also highly specific in response to identity and state of both plants and herbivores. Our findings indicate that differences in long-term UV exposure, herbivore abundance and developmental stage and phenological state of plants, can lead to the production of unique volatile blends.

Interestingly, some of our findings contradict our initial prediction that volatile emissions will be higher for plants exposed to elevated UV radiation and beetle damage, as the effect on compound classes was variable and reduced emission was also observed for some groups measured. These results also differ from published research regarding the increase or decrease of particular groups of compounds in response to particular stressors, i.e., significant increases in terpenoid emission in relation to elevated UV radiation or fatty acid derivatives in response to herbivore damage was not

found here. A plausible explanation is that previous exposure to environmental factors (such as UV in tunnel house studies) and the presence of previous damage or multiple stressors under field conditions (in herbivory trials) may result in different outcomes from studies under laboratory settings using naïve plants and a single stress factor.

This paper also discusses how abiotic and biotic factors could enhance the competitive advantage of an invasive species, either by increasing VOC emissions that enhance the plant's direct or indirect defence or allelopathic properties; or by reducing VOC emissions to allocate more resources to growth and reproduction. More studies are required to gain a better understanding of how changes in VOC emissions by invasive plants affect native communities.

Measuring volatile production by invasive plants and exploring the factors influencing their emission is a vital topic for future studies since the success of invasive plants in a new environment depends directly on the competitive outcome against native plants and indirectly on the plant's interactions with microbes, herbivores and higher trophic levels, all of which can be mediated by VOCs.

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4.8 Appendix 2: Supplementary information

Appendix 2.1

Table A1. Photosynthetically active radiation (PAR), UV-A and UV-B measured under the films (n = 3).

Variable	20% attenuation	95% attenuation	T-test
PAR (µmol m^{-2} s^{-1})	629 ± 295	933 ± 437	$P = 0.233$
UV-A (uW cm^{-2})	1513 ± 617	63 ± 17	$P < 0.001$
UV-B (uW cm^{-2})	117 ± 42	41 ± 25	$P = 0.008$

Appendix 2.2

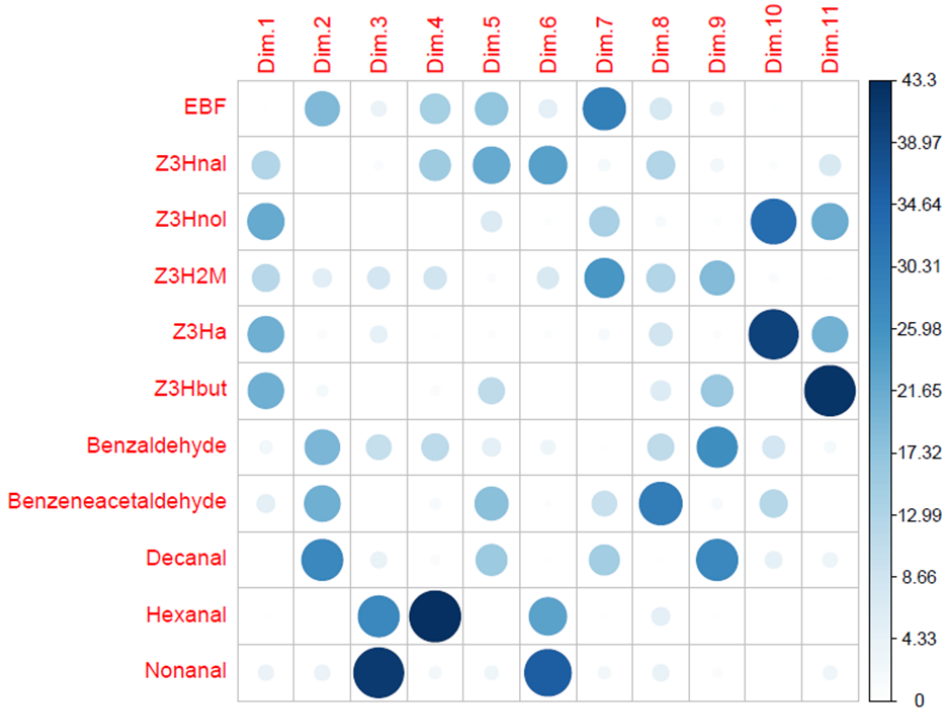

Figure A1. Contributions of variables in PCA based on VOCs identified from heather under different UV-B levels (n = 10 for each treatment). **Abbreviations**: (E)-β-farnesene (EBF), (Z)-3-hexenal (Z3Hnal), (Z)-3-hexenol (Z3Hnol), (Z)-3-hexenyl acetate (Z3Ha), (Z)-3-Hexenyl butyrate (Z3Hbut), (Z)-3-hexenyl 2-methylbutyrate (Z3H2M).

Appendix 2.3

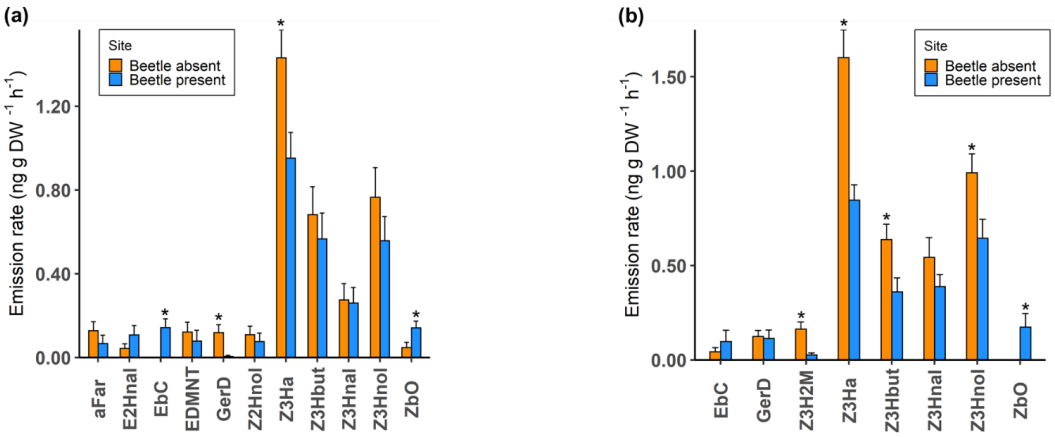

Figure A2. Comparison of main compounds contributing to the observed differences in emissions between sites selected through SIMPER for samples collected on (a) November 2018 and (b) December 2018. Comparisons performed using the Wilcoxon rank sum test (n = 8 for each treatment). **Abbreviations:** (*E*)-2-hexanal (E2Hnal), (*Z*)-3-hexenyl 2-methylbutyrate (Z3H2M), (*E*)-4,8-dimethyl-1,3,7-nonatriene (EDMNT), Germacrene D (GerD), (*E*,*E*)-α-farnesene (aFar), (*Z*)-3-hexenyl acetate (Z3Ha), (*Z*)-3-hexenol (Z3Hnol), (*Z*)-3-hexenal (Z3Hnal), (*Z*)-3-hexenyl butyrate (Z3Hbut), (*Z*)-β-ocimene (Zbo), (*E*)-β-caryophyllene (EbC).

Appendix 2.4

Table A2. VOCs selected through SIMPER as the main compounds contributing to the variations in volatile profiles of heather at second and third instar *L. suturalis* infested and non-infested sites in January 2019. Table shows mean ± SE emission rate (ng gDW^{-1} h^{-1}) of compounds. *P*-values calculated using the Wilcoxon sum rank test and bold fonts indicate compounds that were significantly different between sites (n = 7 for beetle present and 8 for beetle absent).

	Emission rate (mean ± SE)		
Compound	**Beetle absent**	**Beetle present**	***P*-value**
(*E*)-DMNT	0.300 ± 0.210	0.049 ± 0.043	0.396
(*Z*)-2-hexenol	0.497 ± 0.309	0.118 ± 0.065	0.583
(*Z*)-3-hexenol	0.613 ± 0.218	0.544 ± 0.310	0.779
(*Z*)-3-hexenyl 2-methylbutyrate	0.857 ± 0.554	0.298 ± 0.097	0.770
(*Z*)-3-hexenyl acetate	13.280 ± 5.884	2.842 ± 1.155	0.779
(Z)-3-hexenyl benzoate	0.265 ± 0.182	0.173 ± 0.055	0.381
(*Z*)-3-hexenyl butyrate	5.723 ± 4.014	2.093 ± 0.748	0.601
(*Z*)-3-hexenyl valerate	0.527 ± 0.346	0.203 ± 0.072	0.768
(*Z*)-β-ocimene	0.179 ± 0.082	0.977 ± 0.619	0.115
(*E*)-β-caryophyllene	**0.081 ± 0.059**	**0.520 ± 0.226**	**0.008**
Copaene	**0.000 ± 0.000**	**0.390 ± 0.134**	**0.007**
Decanal	0.399 ± 0.208	0.087 ± 0.020	0.779
Epoxylinalol	0.043 ± 0.043	0.167 ± 0.057	**0.058**
Geranyl nitrile	0.362 ± 0.206	0.035 ± 0.030	0.415
Germacrene D	0.113 ± 0.072	0.573 ± 0.377	0.380
Linalool	0.000 ± 0.000	0.587 ± 0.369	**0.057**
Nonanal	0.592 ± 0.233	0.264 ± 0.061	0.536
Octanal	0.082 ± 0.059	0.053 ± 0.010	0.107
Phenylethyl alcohol	**0.000 ± 0.000**	**0.171 ± 0.067**	**0.002**
α-bourbonene	0.118 ± 0.059	0.178 ± 0.112	0.950
(*E*,*E*)-α-farnesene	1.280 ± 0.602	0.109 ± 0.052	0.502
(*E*)-β-farnesene	**0.025 ± 0.024**	**0.072 ± 0.009**	**0.014**
δ-cadinene	**0.042 ± 0.026**	**0.249 ± 0.065**	**0.022**
δ-guaiene	**0.000 ± 0.000**	**0.249 ± 0.063**	**0.001**

Chapter 5

Seasonal and environmental variation in volatile emissions of the New Zealand native plant *Leptospermum scoparium* in weed-invaded and non-invaded sites

Native mānuka plants on the Central Plateau. Photo credits: ***Evans Effah***

This chapter explores the volatile emissions of *Leptospermum scoparium* under plant invasion scenario on the Central North Plateau and determine the environmental variables controlling their emissions. The chapter is presented in the style of the journal *Scientific reports*. The chapter was published by the journal *Scientific reports* as:

Effah, E., Barrett, D.P., Peterson, P.G., Potter, M.A., Holopainen, J.K. and Clavijo McCormick, A. Seasonal and environmental variation in volatile

emissions of the New Zealand native plant *Leptospermum scoparium* in weed-invaded and non-invaded sites. *Scientific reports* 10, 1-11.

Abstract

The New Zealand tea tree *Leptospermun scoparium* (mānuka) is widely known for the antimicrobial properties of its honey. Mānuka is native to New Zealand, growing in a range of environments, including the Central Volcanic Plateau of the North Island, where it is currently threatened by the spread of exotic invasive weeds such as heather (*Calluna vulgaris*) and Scotch broom (*Cytisus scoparius*). Here, we characterise for the first time the aboveground volatile organic compounds (VOCs) produced by mānuka in this area, during summer and winter seasons, in weed-invaded and non-invaded stands. We measured plant volatiles at four sites, each with a distinct combination of woody species: (1) conspecific stands of mānuka; (2) mānuka and another native species (*Dracophyllum subulatum*); and mānuka with one of two European invasive plants, (3) heather or (4) Scotch broom. We also quantified herbivore damage on target mānuka plants and analysed microclimatic variables (soil nutrients, air temperature and soil water content) to investigate their impact on volatile emissions. Our results reveal a strong seasonal effect on volatile emissions, but also significant differences between sites associated with biotic and abiotic changes partly driven by invasive plants. Overall, volatile emission rates from mānuka were typically lower at sites where invaders were present. We point to several factors that could contribute to the observed emission patterns and areas of interest for future research to provide a comprehensive understanding of VOC emissions in nature. Given the vital role of volatile compounds in plant communication,

we also recommend future studies to be performed in multiple seasons, with bigger sample size and more study sites to expand on these findings and explore the ecological impacts of changes in VOC emissions during plant invasion.

Keywords: Exotic invasive species, Indigenous plants, Plant communication, Plant competition, Plant secondary metabolites, Seasonal variation, Volatile organic compounds.

5.1 Introduction

Volatile organic compounds (VOCs), like other plant secondary metabolites, are not directly involved in plant growth, development or reproduction [1], but are vital to facilitate ecological interactions [2]. Such interactions include attracting key pollinators, repelling and deterring herbivores, attracting natural enemies of herbivores and beneficial microbes and shaping plant competition [3-5]. The composition of volatile blends depends on plant species, organ, developmental stage and physiological status of the emitting plant [6-9]. Some plants also adsorb their neighbour's volatiles and passively re-release them [10,11]. However, volatile emission is also extremely plastic, with emissions varying in response to biotic and abiotic factors such as temperature, soil nutrients, herbivory or disease [12]. Species-specificity and environmental plasticity make VOCs an excellent source of information for other organisms, influencing the foraging behaviour of pollinators, herbivores and their natural enemies, and the competitive decisions of nearby plants [13,14].

There is a growing body of evidence showing that VOC emissions depend on the species composition of neighbouring plants and associated environmental changes [15,16]. In plant invasion scenarios, it has been suggested that not only VOC emissions, but the response of the surrounding organisms to the emitted compounds, depends on whether the emitter is a native or exotic plant [17]. Moreover, previous studies suggest that exotic invasive species can recognise neighbours and adjust their metabolic responses accordingly. For example, the invasive species *Centaurea maculosa* accumulated higher levels of defence-related secondary metabolites and lower levels of primary metabolites when growing with conspecifics versus heterospecifics [18].

The chemical behaviour of native plants in environments invaded by exotic species has rarely been studied. However, some native plants can outcompete or persist and coevolve with their invasive counterparts [19,20], suggesting that natives are not necessarily passive during an exotic weed invasion.

The New Zealand tea tree *Leptospermun scoparium* (mānuka in Māori), is a member of the Myrtaceae family and is native to New Zealand and Australia. Mānuka is widely known for the antibacterial, antiviral and anti-inflammatory properties of honey produced from its nectar, which have been subject to extensive research [21,22], suggesting the plant is a prolific producer of secondary metabolites. Mānuka is the most widely distributed, abundant, and environmentally tolerant member of New Zealand's woody flora and is capable of enormous environmental plasticity [23].

Mānuka is a mid-successional species but persists in some areas that do not support succession to climax forest. This includes some low nutrient and poorly drained sites on the Central Volcanic Plateau of the North Island in New Zealand [24]. Another mid-successional dominant native woody species adapted to similar environments in this area is *Dracohyllum subulatum* [25,26]. However, the survival of these and other low growing sub-alpine species on the Central Plateau is threatened by the spread of invasive plants such as heather (*Calluna vulgaris*) and broom (*Cytisus scoparius*). Both heather and broom are woody shrubs, introduced from Europe by early European settlers. Heather was intentionally introduced to Tongariro National Park (which lies within the Central Plateau) in 1912 and has now spread through most of the park and beyond its boundaries, while broom invasion only began in the 1960s and is not yet widespread [27,28].

Analyses of the essential oils of mānuka indicate that this plant produces an array of secondary metabolites, being rich in sesquiterpenes [29,30]. New Zealand and Australian populations showed differences in their essential oils, with oils from Australian plants having significantly more monoterpenes than those in New Zealand [29]. However, the chemical ecology of mānuka remains largely unknown, and no previous study has investigated the plant's volatile emissions (scents) or the factors accounting for their natural variation. Given the ecological importance of VOCs, this information is increasingly relevant as invasive weeds threaten the distribution of this species in its native range.

This study aimed to investigate the natural variation in volatile emissions of mānuka and to identify the factors regulating their emissions. This was done by selecting four sites on the North Island Central Plateau in New Zealand. Each site was distinct and characterised by the presence of mānuka in combination with conspecifics or one of three woody species; *Dracophyllum* (native), heather or broom (both exotics invaders). We measured VOCs in the headspace of target mānuka plants in both summer and winter, quantified herbivore damage on the target plants and collected microclimatic data (soil properties, environmental temperature and soil water content) from each site, to establish the effect of biotic and abiotic variables on VOC emissions.

5.2 Material and methods

5.2.1 Site description and experimental setup

The study was conducted from late November 2017 to September 2018, covering the summer and winter seasons. Four study sites (≥ 50 m × 50 m per site) with distinct plant combinations were selected in the Waiouru Military Training Area without manipulating any variables (Appendix 3.1). Mānuka and *Dracophyllum* are common native woody perennials occurring naturally in the area, while both heather and broom were introduced from Europe. The sites differed in the dominant woody perennials present, with one site having predominantly mānuka plants (from now on referred to as Mānuka - Mānuka or MM), another site having a combination of predominantly mānuka and *Dracophyllum* (Mānuka - *Dracophyllum* or MD). The third site had a combination of predominantly mānuka and heather (Mānuka – Heather or MH), and the last site was having a combination of predominantly mānuka and broom (Mānuka – Broom or MB) (Appendix

3.1). Five replicates consisting of similar-sized mānuka plants were selected at each site. At each site, the positioning of the selected replicates covered ~25 m × 13 m of the respective sites (Appendix 3.1), with about 0.5 m between target paired plants. During each season, data were collected from all sites and VOCs were collected from the same target mānuka plants.

5.2.2 Measuring volatile emissions of mānuka

Aboveground VOCs of mānuka plants of similar size and phenology were collected at each site using the 'push-pull' headspace sampling technique and analysed following the protocol described in a previous study [16]. A similar amount of foliage of sampled plants was enclosed in new oven bags, and carbon-filtered air simultaneously pushed into the bags through a PTFE tube (1.70 L/min) and pulled out (1.20 L/min) through another tube using a portable PVAS22 (Volatile Assay Systems Rensselaer NY). Volatiles in the headspace air was trapped onto a collection filter with 30 mg HayeSep Q adsorbent (Volatile Assay Systems Rensselaer NY) inserted in the pull tube. VOCs were simultaneously collected from different sites at a time to minimise the effect of collection time. Volatiles from each sampled plant was collected for two hours, after which the enclosed foliage was excised and oven-dried at 60º C for 72 hours to calculate emissions per dry weight.

Volatile collection filters were eluted using 200 µL of 95% hexane with 10 ng/mL nonyl acetate ($C_{11}H_{22}O_2$) (Sigma Aldrich) and collected samples analysed using gas chromatography coupled to mass spectrometry. The GC-MS operation conditions and identification of compounds followed the same protocol described in [16]. VOCs were measured from the same target plants

in summer (6 – 13 December 2017) and winter (26 August to 11 September 2018) under similar meteorological conditions.

5.2.3 Examining herbivore damage on mānuka

Using a handheld magnifying glass, visible herbivore damage was examined on the same foliage used during the VOC collections. The number of damage marks on foliage was counted and divided by the dry weight (g) of foliage to estimate damage counts/g as described in [16].

5.2.4 Microclimatic measurements

To measure the soil properties of study sites, we took 20 soil cores (15cm deep × 3 cm diameter) from each (i.e. 4 cores around each paired plants). We measured the fresh weight of soil and determined soil water content (SWC) after oven drying at 40º C until constant weight. Dried soil from each site was then homogenised to represent the average for respective sites and used for nutrients analyses. Soil pH, total carbon (C), total nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na), and soil temperature were measured as described in [16].

Ambient air temperature was recorded by installing temperature data loggers (Tinytag, Gemini) 50 cm above ground level at each site ten days prior to VOC measurements and collected on the last day of VOC measurements for each season.

5.2.5 Data analysis

All statistical analyses were performed using R (version 3.6.2).

Major volatile classes were transformed by (log10x + 1) and compared between the two sampling seasons using linear models ("lm" function in R).

Before performing linear models, total monoterpenoids and sesquiterpenoids rates were normalised to standard temperature (30° C) using an empirically derived coefficient (0.09) as recommended by Guenther and colleagues [31].

The composition of volatile blends produced by mānuka plants was compared between the four sites using a permutational multivariate analysis of variance (PERMANOVA) [32]. PERMANOVA was performed using the vegan package. When there were significant differences between the sites, multiple comparisons were performed using the "pairwise.adonis" function and similarity percentage analysis (SIMPER) used to identify the volatile compounds accounting for the differences between sites [33]. The patterns in VOC emissions between sites were visualised using non-metric multidimensional scaling (NMDS), also with the vegan package. Both PERMANOVA and NMDS were based on Bray-Curtis dissimilarities using square root transformed VOCs data.

Herbivory, soil and ambient temperatures and SWC data were analysed using ANOVA or non-parametric Kruskal-Wallis, and when significant, followed by the Tukey HSD and Mann Whitney tests respectively for multiple comparisons.

We then investigated the potential effects of environmental variables on the VOCs selected through SIMPER using PERMANOVA based on Euclidean distances. PERMANOVA with Euclidean distance produced the classical univariate *F*-statistic, but robust to the assumption of normality and *P*-values obtained by permutation [34,35]. Each model had one response variable (one volatile compound) and environmental factors (soil nitrogen, soil water

content, herbivory and ambient temperature) as predictors. These predictors were selected based on previous reports on their effects on biogenic volatile organic compounds emission [7,12,36]. All response variables were square-root transformed before modelling.

5.3 Results

5.3.1 Seasonal variation in volatile emissions

More volatile compounds were identified from the headspace of mānuka in summer (51 compounds) than in winter (34). VOCs identified from mānuka were classified into their respective chemical groups (Appendix 3.2 and Appendix 3.3) and compared between the two seasons using linear models. The results show significantly lower emissions in winter for green leaf volatiles ($F_{1,38}$ = 103.40, P < 0.001), sesquiterpenoids ($F_{1,38}$ = 24.91, P < 0.001), monoterpenoids ($F_{1,38}$ = 17.03, P < 0.001), aldehydes ($F_{1,38}$ = 23.02, P < 0.001), other esters ($F_{1,38}$ = 11.23, P = 0.002) and total volatile emissions ($F_{1,38}$ = 52.33, P < 0.001, Fig.1).

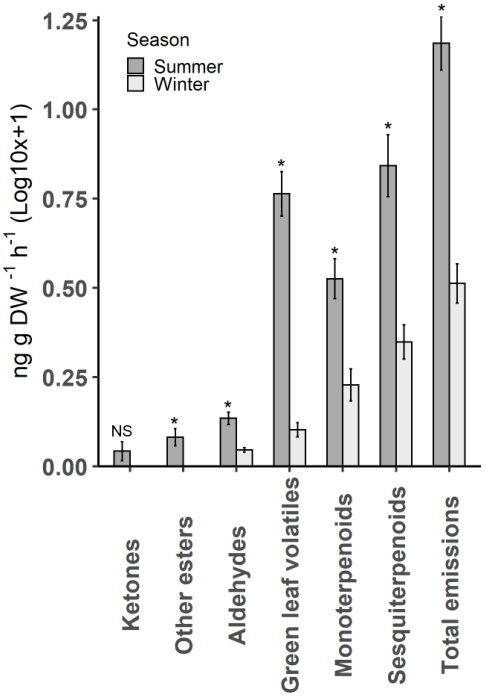

Figure 1. VOC classes for mānuka between the two sampling seasons (n = 20 for each season). Bars show mean ± SE of respective chemical classes. Asterisks (*) indicate a significant difference in emission between seasons and 'NS' means non-significant difference.

5.3.2 Site variation in volatile emissions

In summer, we detected significant variations in the volatile profile of mānuka between the four sites (PERMANOVA; Pseudo-F = 3.71, P < 0.001, Fig. 2). Volatile composition was significantly different between the conspecific stands and the mānuka – heather (Pseudo-F = 7.40, P = 0.012) or mānuka – broom (Pseudo-F = 7.49, P = 0.006, Fig. 2) sites. Difference was also significant between the sites where mānuka occurs with the two invasive plants (Pseudo-F = 3.62, P = 0.009, Fig. 2). Variations between the mānuka – *Dracophyllum* site and other sites were not significant (Fig. 2, Appendix 3.4). The similarity percentage analysis revealed that 27 volatile

compounds accounted for the observed pattern in volatile composition (Fig. 2b).

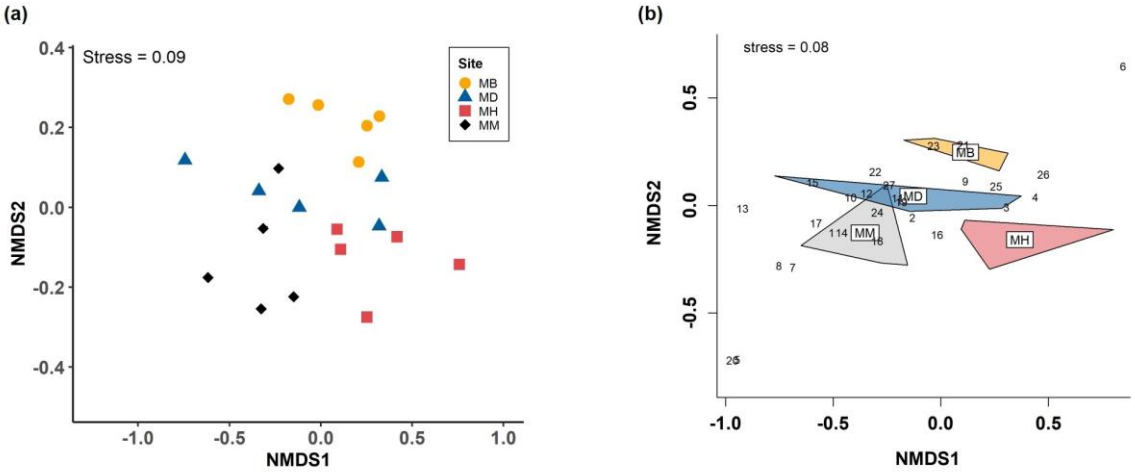

Figure 2. NMDS plots for VOCs emitted by mānuka at four different sites in summer. (A) Based on all the 51 VOCs identified from mānuka and (B) based on 27 VOCs with high contributions selected through SIMPER. The numbers on the graph represent the following compounds; (1) (*E*)-β-caryophyllene, (2) (*Z*)-3-hexenol, (3) (*Z*)-3-hexenyl acetate, (4) (*Z*)-β-ocimene, (5) (*Z*,*E*)-α-farnesene, (6) 2-heptanone, (7) 3-methyl-1-butanol acetate, (8) 2-methyl-1-butanol acetate, (9) aromadendrene, (10) cadinadiene-1,4, (11) calamenene, (12) copaene, (13) germacrene D, (14) humulene, (15) isoledene, (16) lemonol, (17) linalool, (18) o-cymene, (19) α-cubebene, (20) (*E*,*E*)-α-farnesene, (21) α-pinene, (22) α-selinene, (23) β-chamigrene, (24) β-elemene, (25) β-myrcene, (26) β-pinene, (27) β-selinene. **Abbreviations**: Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka– Heather (MH), Mānuka – Mānuka (MM).

The bouquet of volatiles produced by mānuka at the four sites also varied in winter (PERMANOVA; Pseudo-*F* = 2.05, *P* = 0.023, Fig. 3). The pairwise comparison revealed a significant variation between the conspecific stands and the site where mānuka and heather were the dominant species (Pseudo-*F* = 3.46, *P* = 0.023, Fig. 3). Again, VOCs varied significantly between the

sites where mānuka occurs with the two invasive plants (Pseudo-*F* = 5.34, *P* = 0.005, Fig. 3). VOC composition did not vary between the mānuka – *Dracophyllum* site and the other three sites, although borderline significance was found for this site and the mānuka – heather site (Pseudo-*F* = 1.98, *P* = 0.050, Fig. 3, Appendix 3.4). The similarity percentage analysis showed that 23 volatile compounds contributed to the variations detected in VOC emissions between the four sites in winter (Fig. 3b).

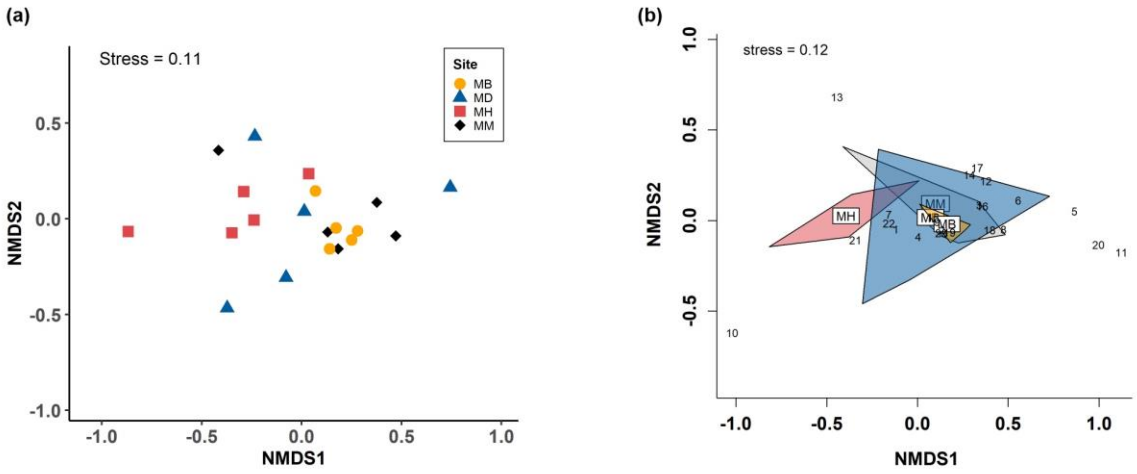

Figure 3. NMDS plots for VOCs emitted by mānuka at four different sites in winter. (A) Based on all the 34 VOCs identified from mānuka and (B) based on 23 VOCs with high contributions selected through SIMPER. The numbers on the graph represent the following compounds; (1) (*E*)-α-bergamotene, (2) (*E*)-β-caryophyllene, (3) (*Z*)-3-hexenal, (4) (*Z*)-3-hexenyl acetate, (5) (*Z*)-β-ocimene, (6) alloaromadendrene, (7) aromadendrene, (8) cadinadiene-1,4, (9) calamenene, (10) eucalyptol, (11) isoledene, (12) limonene, (13) o-cymene, (14) ylangene, (15) α-cubebene, (16) α-gurjunene, (17) α-pinene, (18) α-selinene, (19) β-chamigrene, (20) β-elemene, (21) β-myrcene, (22) β-pinene, (23) β-selinene. **Abbreviations:** Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka – Heather (MH), Mānuka – Mānuka (MM).

5.3.3 Biotic and abiotic factors differ between sites

In summer, there was significantly higher herbivore damage on mānuka in the conspecific stand compared to the other sites (Kruskal-Wallis; X^2 = 13.524, df = 3, P = 0.004, Fig. 4a). However, herbivore damage on mānuka did not differ between the four sites in winter (Kruskal-Wallis; X^2 = 1.250, df = 3, P = 0.741, Fig 4b).

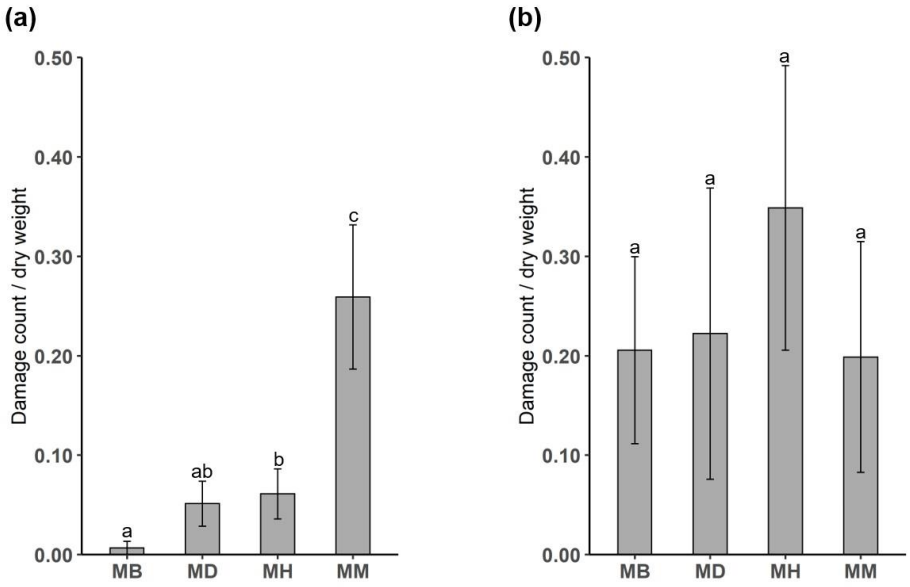

Figure 4. Herbivore damage on mānuka foliage expressed as mean ± SE damage counts/g in summer 2017 (A) and winter 2018 (B). N = 5 for all treatments at each site in both seasons. Different letters indicate a significant difference between sites. **Abbreviations:** Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka – Heather (MH), and Mānuka – Mānuka (MM).

There were significant differences in ambient daytime temperatures between the four sites (Fig. 5a) in both seasons (Kruskal-Wallis; X^2 = 140.50, df = 3, P < 0.001 and X^2 = 129.270, df = 3, P < 0.001 for summer and winter respectively).

Overall, soils from the four study sites had low levels of macro and micronutrients. Soil from broom- and heather-invaded sites had slightly higher levels of nitrogen, carbon and organic matter. Also, soil collected from the broom-invaded site was marginally higher in calcium and magnesium in both seasons (Table 1).

Soil temperature between the sites did not differ in summer (ANOVA; $F_{3,16}$ = 2.462, P = 0.099) but was significantly different in winter (ANOVA; $F_{3,16}$ = *7*.021, P = 0.003), with the mānuka – heather site having the lowest soil temperature (Fig. 5b).

Soil water content also differed significantly between the study sites in summer (Kruskal-Wallis; X^2 = 12.440, df = 3, P = 0.006) and winter (ANOVA; $F_{3,16}$ = 31.840, P < 0.001). The Mānuka – Heather site had significantly higher amounts of SWC than the other sites in both seasons (Fig. 5c).

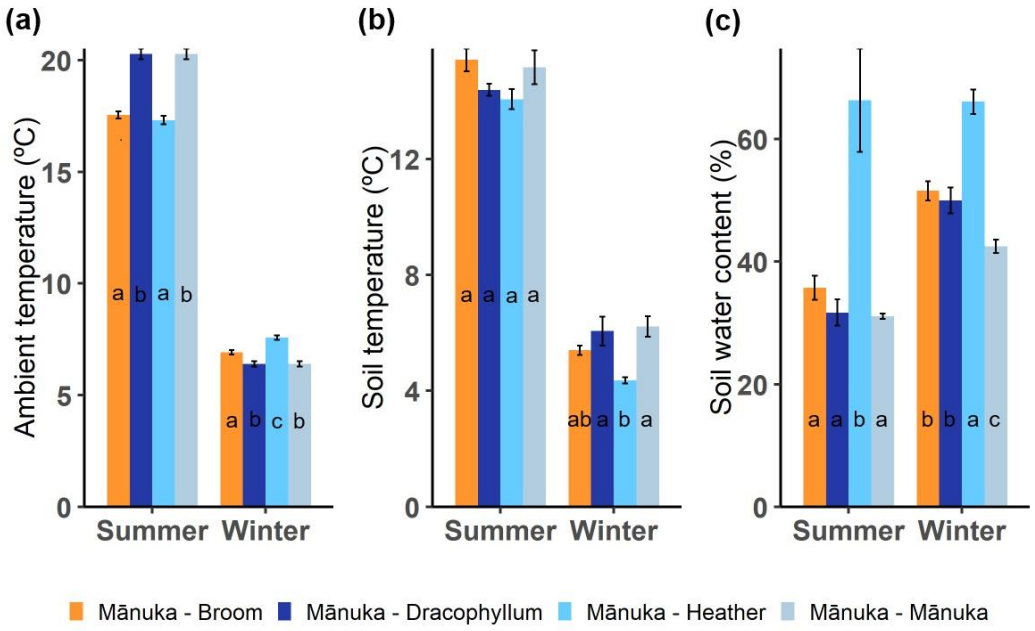

Figure 5. Comparison of (A) ambient temperature (B) soil temperature and (C) soil water content between study sites. Bars show mean ± SE of measured variables, and different letters indicate significant differences between sites.

Table 1. Level of nutrients found in soils from study sites. 20 soil cores collected from each site was homogenised and used for the soil analysis of respective sites. Reference (medium range) represents Hills' laboratories' crop guides for mixed pasture. **Abbreviations:** MB (Mānuka – Broom), MD (Mānuka – *Dracophyllum*), MH (Mānuka – Heather) and MM **(**Mānuka – Mānuka).

	Summer 2017				**Winter 2018**				
Soil Properties	**MB**	**MD**	**MH**	**MM**	**MB**	**MD**	**MH**	**MM**	**Reference (medium range)**
Total nitrogen (%)	0.37	0.26	0.30	0.27	0.40	0.29	0.33	0.26	0.30 – 0.60
Total carbon (%)	4.70	3.40	6.00	3.40	5.50	4.80	6.50	4.00	NA
Phosphorus (me/100g)	8.00	3.00	3.00	3.00	3.00	2.00	3.00	2.00	20 - 30
Sodium (me/100g)	0.08	0.06	0.06	0.09	0.07	0.07	0.07	0.08	0.20 – 0.50
Magnesium (me/100g)	0.72	0.54	0.31	0.56	0.80	0.72	0.42	0.77	1.00 – 1.60
Calcium (me/100g)	3.00	2.20	1.60	2.20	3.70	3.00	2.00	3.10	4.0 – 10.0
Potassium (me/100g)	0.24	0.21	0.24	0.23	0.25	0.26	0.32	0.28	0.40 – 0.60
Organic matter (%)	8.10	5.90	10.4	5.80	9.50	8.30	11.20	6.80	7.0 – 17.0
pH	6.00	6.00	5.70	6.00	5.80	5.80	5.70	6.00	5.80 – 6.20

me/100g = Milliequivalents/100g **NA** = not applicable

5.3.4 Influences of biotic and abiotic factors on volatile emissions

We investigated the effects of some environmental factors known to influence biogenic volatile organic compounds emission using PERMANOVA based on Euclidean distances. In all models, herbivory, ambient daytime temperature, nitrogen (as a proxy for soil nutrients) and soil water content (SWC) were used as predictors while the VOCs selected through SIMPER were response variables (Fig. 2b and 3b).

In summer, the emissions of 15 out of 27 VOCs were significantly affected by at least one of the tested environmental variables, with temperature, herbivory and nitrogen being major drivers. On the other hand, 12 volatile compounds (all terpenoids) were not affected by any of the tested variables (Appendix 3.5). Differences in temperatures between sites significantly affected the emission of ten compounds (*Z*)-3-hexenol, cadinadiene-1,4, germacrene D, humulene, isoledene, α-selinene, β-selinene, 2-methyl-1-butanol acetate and 3-methyl-1-butanol acetate (Table 2). Herbivore damage was the second most important factor, having a significant effect on the emissions of eight compounds, (*Z*)-3-hexenol, copaene, germacrene D, α-cubebene, β-elemene, 2-methyl-1-butanol acetate and 3-methyl-1-butanol acetate (Table 2). Lastly, differences in soil nutrients (N) between sites had a significant effect on the emissions of four compounds, (*Z*)-3-hexenyl acetate, β-pinene, α-selinene and 2-heptanone (Table 2). No compounds were impacted by soil water content.

In winter, differences in emissions of ten sesquiterpenes between the four study sites were explained by the tested environmental variables, while thirteen

compounds were not significantly affected by any of the predictor variables (Appendix 3.5). Temperature differences between the sites affected the emission of aromadendrene, β-chamigrene, β-elemene and β-selinene (Table 2) The emission of cadinadiene-1,4, calamenene, alloaromadendrene and α-gurjunene were significantly affected by differences in soil water content between sites (Table 2). Similarly, differences in soil nutrients accounted for emissions of aromadendrene, α-gurjunene and α-selinene, while herbivore damage affected only the release of cadinadiene-1,4 and isoledene (Table 2).

Table 2. Effects of environmental variables on the emission of VOCs selected through SIMPER for both summer and winter. Pseudo-*F* (*F*) and *P*-values (*P*) calculated using PERMANOVA based on Euclidean distances. Significant *P*-values (P < 0.050) are highlighted in bold.

Compound	**Environmental variables**							
	Herbivory		**Temperature**		**Nitrogen**		**SWC**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Summer 2017								
(*Z*)-3-hexenol	5.140	**0.042**	10.102	**0.008**	0.040	0.840	0.410	0.487
(*Z*)-3-hexenyl acetate	3.810	0.076	1.360	0.259	5.540	**0.038**	0.020	0.898
β-pinene	0.130	0.730	1.608	0.231	7.892	**0.014**	0.209	0.575
Cadinadiene-1,4	2.923	0.089	4.333	**0.041**	0.737	0.410	0.007	0.915
Copaene	5.118	**0.043**	2.051	0.174	2.678	0.110	0.084	0.768
Germacrene D	11.302	**0.005**	14.951	**0.008**	1.449	0.247	0.0015	0.978
Humulene	3.526	0.061	5.648	**0.026**	0.498	0.487	0.389	0.520
Isoledene	0.509	0.431	6.160	**0.014**	0.383	0.552	0.007	0.924
α-cubebene	5.970	**0.023**	2.942	0.116	0.510	0.479	0.062	0.823

α-selinene	0.186	0.645	4.336	**0.048**	4.678	**0.041**	0.405	0.470
β-elemene	15.174	**0.006**	4.410	0.062	0.016	0.904	0.024	0.862
β-selinene	0.970	0.291	4.553	**0.042**	3.213	0.100	0.007	0.920
2-Heptanone	1.231	0.178	1.772	0.228	8.375	**0.014**	0.003	0.934
2-methyl-1-butanol acetate	22.426	**0.002**	6.321	**0.024**	0.572	0.469	1.150	0.262
3-methyl-1-butanol acetate	23.687	**0.001**	6.605	**0.015**	0.444	0.510	0.827	0.405
Winter 2018								
Aromadendrene	<0.001	0.987	8.997	**0.011**	15.823	**0.003**	3.553	0.062
Cadinadiene-1,4	4.945	**0.045**	1.971	0.173	3.871	0.060	5.951	**0.036**
Calamenene	2.995	0.115	3.764	0.077	0.354	0.557	4.594	**0.045**
Isoledene	6.019	**0.036**	1.951	0.198	0.011	0.917	0.348	0.552
Alloaromadendrene	0.747	0.369	0.055	0.816	1.852	0.171	6.498	**0.025**
α-gurjunene	3.386	0.087	1.862	0.181	4.788	**0.045**	7.819	**0.021**
α-selinene	0.764	0.395	2.021	0.165	4.254	**0.048**	4.167	0.053
β-chamigrene	3.412	0.080	8.421	**0.011**	1.880	0.207	2.356	0.156
β-elemene	0.033	0.859	7.424	**0.015**	0.370	0.547	0.667	0.436
β-selinene	2.355	0.127	9.163	**0.010**	1.236	0.262	2.747	0.118

SWC = soil water content

5.4 Discussion

Our study reveals that the New Zealand native plant mānuka is a rich producer of terpenoids mostly sesquiterpenoids. Moreover, the study shows a natural seasonal and site-specific variation in the volatile emissions of this species. Emissions were often lower at sites where the exotic invasive weeds were present (Appendix 3.2 and Appendix 3.3). The variable emission of most volatile compounds was explained by differences in air temperature, herbivory, soil

nitrogen and soil water content levels between sites, but the observed effects were seasonal, being more pronounced during summer. This is consistent with previous reports showing effects of temperature, herbivory, soil nitrogen and soil water content on volatile emissions [7,12,36].

Volatile emission had a clear seasonal pattern, mainly related to temperature. Elevated temperature is known to increase emissions of biogenic volatile organic compounds [7,37]. Temperature can directly affect VOC emissions by regulating the evaporation and release of compounds [38,39]. It can also control stomatal conductance, activities of enzymes and production of photosynthetic metabolites, which can all influence the emission of plant volatiles [40]. Among the environmental variables tested in our study, temperature differences between the sites affected the emission of several volatile compounds, including a green leaf volatile, terpenoids and other esters. Overall, temperatures were higher in the summer and emissions of all major VOCs groups increased at this time of the year. This observation supports the claim that current warming is likely to increase the global emissions of plant volatiles, which can affect their physiological and ecological functions [41].

Herbivory is the most studied biotic factor concerning biogenic volatile emissions. In the field, plants are subjected to attack by numerous herbivores, and they have evolved a variety of defence mechanisms in response. Chemically, many stored volatile organic compounds are released by plants into the atmosphere when damaged by herbivores, but some compounds are also synthesised *de novo* when plants are under herbivore attack [42-44]. Herbivore

loads and their impact vary with the season, as reflected by our data. During summer we observed the most damage to foliage on mānuka in the conspecific stands, and this was probably caused by the high numbers of mānuka beetles (*Pyronota festiva*). At other sites where mānuka occurs with the native species *Dracophyllum* there was less damage despite mānuka beetle numbers being high (Appendix 3.6). We did not investigate the factors accounting for the less damage at this site. However, possible reasons could include higher defence responses such as the increased emissions of some monoterpenoids by mānuka at this site (Appendix 3.2 and Appendix 3.3) or relatively recent migration of the beetles to this site at the time of the experiment. In contrast, at sites where mānuka occurs with broom and heather, both damage levels and mānuka beetle numbers were low (Fig. 4, Appendix 3.6). This may indicate a disruption in communication between mānuka and its principal herbivore and suggests that the abundance of herbivorous arthropod is reduced at sites where invasive plants are dominant [16,45].

In summer, herbivory on mānuka accounted for the emissions of (*Z*)-3-hexenol, copaene, germacrene D, α-cubebene, β-elemene and some esters. This herbivore-induced volatiles has been identified as key elements of plant defence against herbivores [3,46-48]. In contrast, the effect of herbivore damage on VOC emissions was almost negligible in winter when herbivores were mostly absent. This suggests that in summer, when there is a higher threat of herbivory, plants could benefit by emitting higher amounts of VOCs to directly repel herbivores and attract their natural enemies [49-52]. Due to the small number of arthropods

identified at all sites in winter (data not shown), it is possible high foliage damage recorded in this season is cumulative and occurred in preceding seasons. Other factors, like cold-stress, may also impact VOC emissions in winter, and we recommend more studies to investigate plant volatile emissions in cold environments.

Invasive woody species are known to impact the microclimate of the sites they invade, reducing the direct effects of high radiation and temperature, increasing water availability and causing accumulation of nutrients and organic matter in the soil [53,54]. For example, soil from heather stands in the Tongariro National Park in New Zealand was extremely acidic and had a high level of carbon and nitrogen compared with soils from natives' stands [55]. Several studies assessing the impact of broom invasion on soil properties have also reported increased levels of organic matter, C, N and P at invaded sites [56-58]. This is consistent with our results, where we found lower ambient temperatures in the invaded sites during summer, higher water availability in the sites invaded by heather, and higher carbon, nitrogen, and organic matter contents in both heather- and broom-invaded sites. Among soil nutrients, N is the most studied in relation to VOC emissions, and effects are plant species and compound dependent [7,59]. We found a significant effect of N on the emission of some VOCs in both summer and winter, suggesting potential changes in the plant's biochemistry resulting from modification in soil composition by the invasive species. To improve our knowledge of the relationship between exotic weeds and soil chemistry of the new habitat, we suggest further studies to test whether differences in soil

properties often reported during plant invasion are caused by the presence of exotic weeds rather than the invaders' preference to grow at local nutrient sites.

Our results also show an effect of differences in soil water content between the sites on emission of the sesquiterpenes cadinadiene-1,4, calamenene, alloaromadendrene and α-gurjunene. This effect of soil water content on VOC emissions was not detected in summer, which suggests that under natural conditions plants may give priority to certain stressors such as higher temperature and herbivory in summer. In addition, the effect of water availability on plant volatile emissions may vary depending on the severity and duration of the stress, with opposing results in the literature [36,40].

Previous studies have reported variation in volatile emissions between conspecific- and heterospecific stands. For instance, *Pinus halepensis* reduced its VOC emission when sharing a pot with *Quercus ilex* compared with conspecifics [60]. In the field, the Mediterranean plant *Rosmarinus officinalis* also reduced its emission of monoterpenes when neighbored with *Pinus halepensis* [61]. A recent study also showed that the invasive plant *Calluna vulgaris* produces lower amounts of volatiles at a site where it co-exists with another invasive plant *Cytisus scoparius*, which is a nitrogen-fixer, capable of modifying soil properties [16]. However, other studies also show lower emissions when plants were paired with conspecifics [62]. In the present study, we found that VOC emissions by mānuka were often lower at sites where mānuka co-occurred with heterospecifics, particularly with invasive plants (Appendix 3.2 and Appendix 3.3).

Considering the limitation of small sample size on the present study, we recommend further studies to investigate whether reductions in VOC emissions by plants in heterospecific stands is widespread and whether the co-evolutionary history between neighbouring plants influences emissions. Such studies should include more study sites and perform experiments for much longer periods, covering different developmental stages, age of target plants and changes in other conditions in study sites. Another important aspect to consider in future studies is the genetic relatedness of plants in conspecific- and heterospecific stands since VOC emissions and their impact can vary between close and distance relatives [63,64]. In the present study, it is possible that mānuka plants at a site are more closely related thereby producing unique volatile blend, which may [65] or may not [64] affect their ecological roles. Therefore, we recommend future studies to perform a detail genetic analysis of plants to ensure that changes in VOC emissions are not merely reflecting the genetic variability of tested plants.

5.5 Conclusion

We have, for the first time, characterised the volatile emissions of mānuka plants and provided evidence for natural variation in VOC emissions. Our results show that variability in the emission of most compounds produced by mānuka is influenced by microclimatic factors and herbivory, with strong seasonal differences. Our study shows that temperature is a significant factor influencing VOC emissions, suggesting that current warming is likely to increase the global emissions of plant volatiles and affect their ecological roles. Although different

biotic and abiotic factors explained emissions of most compounds, there were also VOC emissions that were not explained by these variables, yet their relative proportions varied between the sites (Appendix 3.2 and Appendix 3.3). Therefore, further studies are needed to investigate other factors that may influence VOC emissions such as genetic variation in plants, plant community effects, belowground herbivory or association with beneficial microbes or pathogens.

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5.8 Appendix 3: Supplementary information

Appendix 3.1

Table A1. GPS coordinates for experimental sites

Site / dominant woody species	Location (GPS coordinates)	Sampled area
Mānuka - Broom	Long. 175.6685 – Lat. -39.451283	23 m × 11 m
Mānuka - *Dracophyllum*	Long. 175.685483 – Lat. -39.432933	24 m × 12 m
Mānuka - Heather	Long. 175.734317 – Lat. -39.314683	24 m × 13 m
Mānuka - Mānuka	Long. 175.685483 – Lat. -39.432933	27 m × 14 m

Appendix 3.2

Table A4. Emission rates of VOCs identified from the headspace of mānuka at four different sites in summer. Comparison performed using generalised linear model assuming Gamma distribution (log-link) with VOCs as response and sites and predictor variables. Likelihood ratio test used to test the significance of predictor. Prior to modeling, a small constant (0.0001) was added to all response variables. P-values in bold font indicates significant difference ($P < 0.05$). N = 5 replicates for each site.

	Mean ± SE emission rate per site (ng $gDW^{-1}h^{-1}$)				Likelihood ratio test		
Compound	**MM**	**MH**	**MD**	**MB**	X^2	**DF**	***P*-value**
Green leaf volatiles							
(*E*)-2-hexenal	0.08 ± 0.05	ND	0.05 ± 0.04	ND	28.97	3	< **0.001**
(*Z*)-3-hexenol	0.90 ± 0.24	0.14 ± 0.04	0.77 ± 0.37	0.18 ± 0.05	18.08	3	< **0.001**
(*Z*)-3-hexenyl acetate[i]	10.23 ± 2.00	2.13 ± 0.54	3.39 ± 0.96	5.56 ± 1.02	16.55	3	**0.001**
Hexanol	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.58	3	0.900
Hexyl acetate	0.24 ± 0.06	0.04 ± 0.02	0.11 ± 0.02	0.14 ± 0.06	5.17	3	0.160
Total	11.47 ± 2.22	2.33 ± 0.58	4.34 ± 1.00	5.80 ± 1.09	17.17	3	< **0.001**
Monoterpenoids							
(*E*)- β-ocimene	0.05 ± 0.01	0.06 ± 0.03	0.02 ± 0.02	ND	18.81	3	<**0.001**
(*Z*)-β-ocimene	0.57 ± 0.45	0.54 ± 0.42	0.29 ± 0.15	0.17 ± 0.04	3.13	3	0.371
Lemonol	0.35 ± 0.14	0.26 ± 0.08	0.40 ± 0.25	0.08 ± 0.05	2.91	3	0.406
Limonene[i]	0.16 ± 0.04	0.05 ± 0.02	0.13 ± 0.05	0.12 ± 0.04	3.54	3	0.316
Linalool[i]	2.77 ± 1.26	0.98 ± 0.27	6.22 ± 4.92	0.18 ± 0.05	18.72	3	< **0.001**
Terpinen-4-ol	0.02 ± 0.01	ND	0.03 ± 0.02	ND	28.76	3	< **0.001**
α-phellandrene	0.03 ± 0.02	ND	0.03 ± 0.02	0.01 ± 0.01	14.33	3	**0.002**
α-pinene[i]	0.29 ± 0.16	0.26 ± 0.14	1.93 ± 1.15	1.46 ± 0.27	7.75	3	0.052
o-cymene	0.36 ± 0.13	0.04 ± 0.02	0.35 ± 0.24	0.05 ± 0.04	5.43	3	0.143
β-myrcene	0.78 ± 0.44	0.51 ± 0.30	0.53 ± 0.16	1.12 ± 0.39	1.47	3	0.690
β-pinene[i]	0.64 ± 0.22	0.35 ± 0.07	0.31 ± 0.04	1.67 ± 0.70	16.89	3	**0.001**
Eucalyptol[i]	0.15 ± 0.09	0.01 ± 0.01	0.08 ± 0.06	0.04 ± 0.02	4.07	3	0.254
Total	6.17 ± 1.84	3.06 ± 1.04	10.33 ± 6.73	4.88 ± 1.31	5.59	3	0.133
Sesquiterpenoids							
(*E*)-α-bergamotene	0.20 ± 0.08	0.09 ± 0.03	0.10 ± 0.03	0.10 ± 0.04	0.99	3	0.803
(*E*)-β-caryophyllene[i]	13.89 ± 8.27	2.11 ± 0.57	10.87 ± 5.10	0.71 ± 0.24	19.38	3	< **0.001**
Alloaromadendrene	0.14 ± 0.02	0.07 ± 0.02	0.13 ± 0.08	0.19 ± 0.08	1.60	3	0.659
Aromadendrene	0.15 ± 0.06	0.07 ± 0.01	0.14 ± 0.13	0.33 ± 0.19	3.21	3	0.360
Cadinadiene-1,4	0.68 ± 0.18	0.09 ± 0.03	0.85 ± 0.54	0.26 ± 0.11	9.82	3	**0.020**
Calamenene	2.62 ± 0.49	0.48 ± 0.12	2.40 ± 1.41	1.31 ± 0.54	10.20	3	**0.017**
Caryophyllene oxide	0.24 ± 0.09	0.02 ± 0.01	0.10 ± 0.06	0.03 ± 0.02	6.14	3	0.105
Copaene	0.53 ± 0.12	0.06 ± 0.03	0.38 ± 0.24	0.25 ± 0.10	5.07	3	0.167
Germacrene D	0.27 ± 0.05	ND	0.20 ± 0.12	0.04 ± 0.03	27.76	3	< **0.001**
Ylangene	0.12 ± 0.05	0.03 ± 0.01	0.13 ± 0.08	0.06 ± 0.02	3.35	3	0.341

α-bisabolol	0.05 ± 0.01	ND	0.04 ± 0.03	ND	36.62	3	< **0.001**
α-cubebene	1.09 ± 0.27	0.21 ± 0.04	0.88 ± 0.47	0.40 ± 0.16	10.29	3	**0.016**
(*E*,*E*)-α-farnesene	2.99 ± 1.61	0.14 ± 0.14	ND	ND	33.79	3	< **0.001**
α-gurjunene	0.13 ± 0.03	0.03 ± 0.01	0.18 ± 0.10	0.15 ± 0.07	5.75	3	0.125
Humulene[i]	0.75 ± 0.42	0.12 ± 0.03	0.67 ± 0.27	0.05 ± 0.01	14.92	3	**0.001**
Isoledene	0.73 ± 0.30	0.05 ± 0.02	1.36 ± 0.90	0.24 ± 0.10	14.59	3	**0.002**
α-selinene	0.22 ± 0.06	0.02 ± 0.01	0.38 ± 0.27	0.23 ± 0.09	10.27	3	**0.016**
β-chamigrene	1.16 ± 0.73	0.43 ± 0.18	4.16 ± 2.65	1.89 ± 0.64	4.10	3	0.250
β-elemene	4.06 ± 1.51	0.66 ± 0.21	3.19 ± 1.83	0.56 ± 0.16	16.72	3	**0.001**
(*E*)-β-farnesene	0.06 ± 0.03	0.03 ± 0.03	0.03 ± 0.03	ND	26.57	3	< **0.001**
(*Z*,*E*)-α-farnesene	0.53 ± 0.32	0.04 ± 0.04	ND	ND	32.50	3	< **0.001**
β-selinene	1.84 ± 0.27	0.26 ± 0.10	2.84 ± 1.73	1.52 ± 0.48	9.90	3	**0.019**
γ-elemene	0.19 ± 0.10	0.01 ± 0.01	0.17 ± 0.10	0.01 ± 0.01	8.96	3	**0.030**
δ-cadinene	0.10 ± 0.06	ND	0.18 ± 0.13	ND	30.13	3	< **0.001**
Total	32.70 ±12.09	4.99 ± 1.27	29.38 ± 15.59	8.32 ± 2.94	14.23	3	**0.003**
Aldehydes							
Decanal	0.07 ± 0.01	0.12 ± 0.04	0.06 ± 0.01	0.15 ± 0.03	11.30	3	**0.010**
Heptanal	0.04 ± 0.03	0.03 ± 0.03	0.04 ± 0.02	0.06 ± 0.04	0.32	3	0.957
Nonanal	0.11 ± 0.02	0.23 ± 0.09	0.10 ± 0.04	0.32 ± 0.06	5.16	3	0.161
Octanal	0.04 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	1.51	3	0.681
Total	0.26 ± 0.03	0.43 ± 0.16	0.24 ± 0.06	0.61 ± 0.11	8.19	3	**0.042**
Other esters							
3-methyl-1-butanol acetate	0.39 ± 0.12	0.04 ± 0.03	0.08 ± 0.03	ND	27.11	3	< **0.001**
2-methyl-1-butanol acetate	0.26 ± 0.09	0.03 ± 0.02	0.06 ± 0.02	ND	26.55	3	< **0.001**
(*Z)*-3-octenyl acetate	0.03 ± 0.01	ND	0.04 ± 0.04	0.01 ± 0.01	12.69	3	**0.005**
Hexyl 2-methylbutyrate	0.14 ± 0.04	0.23 ± 0.02	0.13 ± 0.03	0.23 ± 0.01	1.4319	3	0.698
β-phenethyl acetate	0.05 ± 0.02	ND	ND	ND	101.32	3	< **0.001**
Total	0.73 ± 0.22	0.07 ± 0.05	0.18 ± 0.07	0.01 ± 0.01	11.324	3	**0.010**
Ketones							
2-heptanone	0.01 ± 0.01	ND	ND	0.62 ± 0.40	38.33	3	< **0.001**
Total VOC emissions	51.34 ±14.81	10.89 ± 2.74	44.47 ± 22.49	20.24 ± 4.85	12.50	3	**0.006**

Abbreviations: Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka – Heather (MH), and Mānuka – Mānuka (MM).

[i] Compounds verified by authentic standards

Appendix 3.3

Table A5. Emission rates of VOCs identified from the headspace of mānuka at four different sites in winter. Comparison performed using generalised linear model assuming Gamma distribution (log-link) with VOCs as response and sites and predictor variables. Likelihood ratio test used to test the significance of predictor. Prior to modeling, a small constant (0.0001) was added to all response variables. P-values in bold font indicates significant difference ($P < 0.05$). N = 5 replicates for each site.

	Mean ± SE emission rate per site (ng $gDW^{-1}h^{-1}$)				**Likelihood ratio test**		
Compound	**MM**	**MH**	**MD**	**MB**	X^2	**DF**	***P*-value**
Green leaf volatiles (GLVs)							
(*Z*)-3-hexenal	0.09 ± 0.06	0.02 ± 0.01	0.05 ± 0.04	0.05 ± 0.05	1.13	3	0.770
(*Z*)-3-hexenyl acetate[i]	0.27 ± 0.13	0.11 ± 0.03	0.26 ± 0.14	0.20 ± 0.02	2.37	3	0.499
(*Z*)-3-hexenol	0.04 ± 0.02	ND	0.02 ± 0.02	0.05 ± 0.02	14.05	3	**0.003**
Total GLVs	0.41 ± 0.19	0.13 ± 0.03	0.34 ± 0.20	0.29 ± 0.04	2.96	3	0.398
Monoterpenoids (MTs)							
(*Z*)-β-ocimene	0.10 ± 0.07	0.06 ± 0.04	0.09 ± 0.09	0.06 ± 0.04	0.21	3	0.976
Limonene[i]	0.09 ± 0.02	0.04 ± 0.02	0.28 ± 0.21	0.07 ± 0.02	5.11	3	0.164
Linalool[i]	0.03 ± 0.02	0.03 ± 0.02	0.06 ± 0.05	0.02 ± 0.02	1.14	3	0.769
α-pinene[i]	0.60 ± 0.21	0.24 ± 0.07	5.66 ± 4.36	0.85 ± 0.34	14.46	3	**0.002**
β-myrcene	0.51 ± 0.32	1.20 ± 0.92	0.37 ± 0.17	0.79 ± 0.31	1.28	3	0.735
β-pinene[i]	0.49 ± 0.21	0.46 ± 0.15	0.75 ± 0.30	1.03 ± 0.28	3.67	3	0.299
γ-terpinene	0.04 ± 0.04	0.05 ± 0.05	0.13 ± 0.07	0.03 ± 0.03	1.50	3	0.682
o-cymene	0.19 ± 0.13	0.08 ± 0.05	0.30 ± 0.13	0.04 ± 0.04	2.07	3	0.559
Eucalyptol[i]	0.02 ± 0.02	0.03 ± 0.03	0.84 ± 0.80	0.01 ± 0.01	9.76	3	**0.020**
Total MTs	2.06 ± 0.25	2.18 ± 1.19	8.47 ± 5.11	2.89 ± 0.86	8.71	3	**0.033**
Sesquiterpenoids (SQTs)							
(*E*)-α-bergamotene	0.12 ± 0.04	0.08 ± 0.03	0.22 ± 0.12	0.12 ± 0.03	1.19	3	0.755
(*E*)-β-caryophyllene[i]	0.67 ± 0.42	0.11 ± 0.03	0.43 ± 0.23	0.38 ± 0.08	9.81	3	**0.020**
Alloaromadendrene	0.08 ± 0.04	0.05 ± 0.03	0.07 ± 0.06	0.14 ± 0.04	0.73	3	0.866
Aromadendrene	0.04 ± 0.03	0.11 ± 0.03	0.05 ± 0.04	0.29 ± 0.05	4.59	3	0.204

Cadinadiene-1,4	0.23 ± 0.08	0.04 ± 0.01	0.16 ± 0.10	0.26 ± 0.05	5.08	3	0.166
Calamenene	1.51 ± 0.37	0.37 ± 0.11	1.08 ± 0.66	1.12 ± 0.23	7.96	3	**0.047**
Caryophyllene oxide	0.07 ± 0.04	ND	0.02 ± 0.01	ND	27.38	3	< **0.001**
Copaene	0.26 ± 0.07	0.08 ± 0.02	0.23 ± 0.15	0.21 ± 0.05	6.16	3	0.104
α-selinene	0.38 ± 0.14	0.05 ± 0.01	0.32 ± 0.19	0.53 ± 0.15	6.93	3	0.074
β-chamigrene	2.06 ± 0.51	0.26 ± 0.03	1.79 ± 0.98	1.71 ± 0.19	18.66	3	< **0.001**
β-elemene	0.14 ± 0.05	ND	0.20 ± 0.12	0.08 ± 0.02	21.75	3	< **0.001**
Humulene[i]	0.03 ± 0.03	ND	0.05 ± 0.04	ND	26.08	3	< **0.001**
Isoledene	0.17 ± 0.10	0.01 ± 0.01	0.16 ± 0.13	0.10 ± 0.05	3.6	3	0.308
Ylangene	0.06 ± 0.02	0.06 ± 0.02	0.25 ± 0.22	0.10 ± 0.04	2.79	3	0.424
α-amorphene	0.05 ± 0.04	0.04 ± 0.03	0.04 ± 0.04	0.07 ± 0.06	0.16	3	0.984
α-cubebene	0.46 ± 0.11	0.13 ± 0.05	0.45 ± 0.30	0.26 ± 0.07	7.21	3	0.066
α-gurjunene	0.11 ± 0.03	0.04 ± 0.02	0.12 ± 0.08	0.16 ± 0.02	2.59	3	0.459
Germacrene D	0.05 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.02	1.62	3	0.655
β-selinene	2.42 ± 0.63	0.30 ± 0.01	1.92 ± 0.98	1.77 ± 0.19	18.93	3	< **0.001**
Total SQTs	8.91 ± 2.28	1.76 ± 0.39	7.58 ± 4.39	7.32 ± 0.93	12.56	3	**0.006**
Aldehydes							
Decanal	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	1.37	3	0.713
Heptanal	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	2.20	3	0.532
Nonanal	0.07 ± 0.02	0.08 ± 0.03	0.04 ± 0.03	0.09 ± 0.01	0.68	3	0.879
Total aldehydes	0.12 ± 0.03	0.11 ± 0.03	0.10 ± 0.04	0.13 ± 0.02	0.14	3	0.986
Total VOC emissions	11.49 ± 2.42	4.18 ± 1.57	16.48 ± 9.63	10.63 ±1.17	8.58	3	0.035

Abbreviations: Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka – Heather (MH), and Mānuka – Mānuka (MM).

[i] Compounds varified by authentic standards

Appendix 3.4

Table A2. Pairwise comparison of volatile profile of mānuka between sites. Bold fonts show significant difference.

Pairs	DF	Sum of Squares	Pseudo-*F*	R^2	*P*-value
Summer					
MM vs. MH	**1**	**0.372**	**7.399**	**0.481**	**0.012**
MM vs. MD	1	0.108	1.610	0.167	0.176
MM vs. MB	**1**	**0.305**	**7.494**	**0.484**	**0.006**
MH vs. MD	1	0.177	2.420	0.232	0.080
MH vs. MB	**1**	**0.169**	**3.623**	**0.312**	**0.009**
MD vs. MB	1	0.137	2.147	0.212	0.113
Winter					
MM vs. MH	**1**	**0.258**	**3.557**	**0.308**	**0.035**
MM vs. MD	1	0.065	0.650	0.075	0.679
MM vs. MB	1	0.042	0.777	0.088	0.692
MH vs. MD	1	0.186	1.983	0.199	0.050
MH vs. MB	**1**	**0.269**	**5.540**	**0.409**	**0.012**
MD vs. MB	1	0.099	1.317	0.141	0.234

Abbreviations: Versus (vs.), Mānuka – Mānuka (MM), Mānuka – Heather (MH), Mānuka – *Dracophyllum* (MD), Mānuka – Broom (MB).

Appendix 3.5

Table A3. Effects of environmental variables on emissions of VOCs selected through SIMPER for both summer and winter. *F*-values (Pseudo-F) and *p*-values (*P*) calculated using PERMANOVA based on Euclidean distances. Table shows VOCs whose emissions were not significantly affected by any of the tested variables.

Compound	**Environmental variables**							
	Herbivory		**Temperature**		**Nitrogen**		**SWC**	
Summer 2017	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
(*Z*)-β-ocimene	0.118	0.691	0.066	0.803	0.379	0.527	0.431	0.418
Lemonol	4.244	0.061	0.286	0.597	2.978	0.104	0.095	0.730
Linalool	1.054	0.223	2.574	0.115	0.808	0.408	0.041	0.806
α-pinene	1.557	0.206	0.379	0.536	2.290	0.145	0.324	0.599
o-cymene	2.996	0.089	4.442	0.057	<0.001	0.985	0.219	0.672
β-myrcene	0.230	0.643	0.005	0.935	2.081	0.155	1.170	0.330
(*E*)-β-caryophyllene	3.711	0.080	4.417	0.058	0.403	0.543	0.264	0.583
Aromadendrene	0.011	0.911	0.471	0.503	2.387	0.141	0.051	0.813
Calamenene	3.654	0.083	2.993	0.105	1.217	0.297	<0.001	0.997
(*Z*,*E*)-α-farnesene	2.456	0.093	0.488	0.530	0.001	0.977	0.027	0.814
(*E*,*E*)-α-farnesene	2.453	0.082	0.683	0.424	0.002	0.970	0.012	0.902
β-chamigrene	0.025	0.863	0.712	0.420	0.797	0.393	0.001	0.972
Winter 2018								
(*Z*)-3-hexenyl acetate	0.381	0.554	0.790	0.381	0.160	0.705	0.189	0.676
(*Z*)-3-hexenal	0.381	0.538	0.656	0.414	0.010	0.927	0.019	0.893
(*Z*)-β-ocimene	0.117	0.757	0.001	0.981	0.043	0.853	0.001	0.980
Limonene	1.327	0.242	2.000	0.189	0.028	0.862	1.805	0.158
o-cymene	0.195	0.667	1.369	0.271	0.624	0.467	0.680	0.421
α-pinene	0.053	0.812	1.547	0.250	0.088	0.800	0.006	0.937
β-myrcene	0.030	0.844	0.774	0.369	0.190	0.656	0.347	0.528
β-pinene	0.358	0.571	0.021	0.881	4.148	0.054	1.702	0.202
Eucalyptol	4.952	0.055	2.033	0.171	<0.001	0.997	0.025	0.869
(*E*)-α-bergamotene	0.944	0.339	0.498	0.506	0.070	0.801	1.681	0.203
(*E*)-β-caryophyllene	2.946	0.113	2.360	0.153	0.118	0.763	0.475	0.469
Ylangene	0.591	0.436	0.062	0.823	<0.001	0.997	0.103	0.751
α-cubebene	2.350	0.146	3.399	0.063	0.062	0.832	1.696	0.202

SWC = soil water content

Appendix 3.6

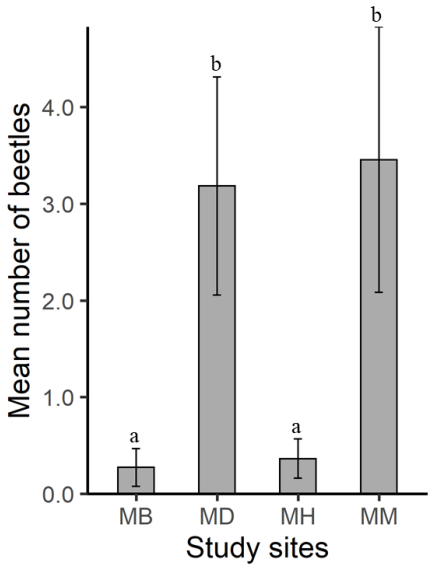

Figure A1. Abundance of mānuka beetles between the four study sites. Beetles were captured using in summer the flight interception traps (n = 3), pitfall traps (n = 3) and by directly beating the target plant foliage on a tray (n = 5). Data was analysed using GLM assuming poisson distribution (log-link). **Abbreviations:** Mānuka – Broom (MB), Mānuka – *Dracophyllum* (MD), Mānuka – Heather (MH), and Mānuka – Mānuka (MM). Different letters indicate significant differences ($P < 0.05$) between sites.

Chapter 6

Effects of two invasive weeds on arthropod community structure on the Central Plateau of New Zealand

Adult mānuka beetles feeding on mānuka plants. Photo credits: ***Evans Effah***

This chapter investigates the effect of plant invasion on the arthropods abundance and distribution on the Central North Plateau, New Zealand. The chapter is presented in the style of the journal *Plants*. The chapter was published by the journal as:

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Abstract

Heather (*Calluna vulgaris*) and broom (*Cytisus scoparius*), originally from Europe, are the main invasive plants on New Zealand's North Island Central Plateau where they pose a threat to native flora and fauna. Given the strong link between arthropod communities and plants, we explored the impact of these invasive weeds on the diversity and composition of associated arthropod assemblages in this area. We collected and identified to order the arthropods in heather-invaded areas, broom-invaded areas, and areas dominated by the native species mānuka (*Leptospermum scoparium*) and *Dracophyllum* (*Dracophyllum subulatum*). During summer and autumn, arthropods were collected using beating trays, flight intercept traps and pitfall traps. We calculated diversity indices (Richness, Shannon's index and Simpson's index) at the order level for our samples, and used permutational multivariate analysis (PERMANOVA) to explore differences in order-level community composition. Our results show that invasive plants did not profoundly impact arthropod order richness, but community composition varied significantly for all trapping methods in both seasons. The presence of broom was associated with an increase in arthropod abundance, while heather was linked with a reduction. Under all possible plant pairings between heather, broom, mānuka and *Dracophylum*, we further explored the impact of neighbouring plant identity on arthropod community composition for the samples collected using beating trays. The results suggest that during plant invasion, arthropod communities can be affected by neighbouring plant identity and that impacts vary between arthropod sampling

methods and seasons. Therefore, sampling sites with different plant community compositions need to be sampled across multiple seasons using a variety of trapping methods if we are to understand better the impacts of invasive weeds on arthropod communities.

Keywords: Arthropod diversity, Arthropod community composition, Plant community composition, Exotic weeds, Invasion ecology, Invasive species

6.1 Introduction

Increased human migration, trade and climate change are significant factors contributing to the spread of plants beyond their natural boundaries [1-3]. Some introduced plants survive, spread, and become invasive in new habitats. A variety of factors contribute to the success of invasive plants in their new environment, including biogeographic affinity between their native and invasive range, rapid and high reproductive outputs [4], rapid growth and high-stress tolerance [5, 6], lack of specialist natural enemies [7], high phenotypic plasticity [8-10], the ability to release phytotoxic compounds into the environment [11], and the potential to rob native plants of their mutualists [12]. The threats posed by exotic invasive plants have gained much attention in recent years, with loss of biodiversity often associated with plant invasion [13, 14].

Invasive plants change the vegetation structure and composition of their new habitats, either through direct competition or modification of the environment [15]. Due to the impact of microclimatic factors on their development and their close interaction with plants, arthropod communities are vulnerable to these

changes. Several studies report a significant decrease in arthropod diversity and abundance in response to plant invasions [reviewed in 15], and others suggest that arthropod assemblages could be restored when invasive plants are eradicated [16-18]. However, arthropods fill diverse niches and ecological roles, and their responses to plant invasion may vary. For instance, some invasive plants may attract pollinators [19], provide alternative resources for generalist herbivores, or create favourable conditions for predators and decomposers [15]. It is therefore important to explore changes in arthropod community composition in different invasion scenarios through the seasons, using a range of sampling techniques to avoid faulty generalisation.

The Central Plateau of New Zealand is widely known for its imposing landscape. Tongariro National Park, which lies within the Volcanic Plateau, was awarded a world heritage site listing in 1993 due to its natural, scientific and cultural significance [20]. However, this ecosystem is threatened by the spread of exotic invasive weeds including heather (*Calluna vulgaris*) and broom (*Cytisus scoparius*), both of European origin. Heather was deliberately planted in the Tongariro National Park by European settlers in 1912 [21] and is now the most widespread weed in the area, while the broom invasion began in the 1960s [22]. The study area was originally covered by subalpine shrubland, tussockland, and montane *Nothofagus* and *Libocedrus* forests, but volcanic activity and forest clearing due to burning have created large areas of tussockland or converted pasture, where only a few woody perennials like *Dracophyllum* (*Dracophyllum subulatum*) and mānuka (*Leptospermum scoparium*) persist [23]. These species

are adapted to unfertile free-draining volcanic ash soils, large temperature extremes and varying rainfall conditions [24-26].

Few studies have reported the impact of invasive plants on the surrounding flora and fauna in this ecosystem. A previous study found that plant communities dominated by tussock and other grasses were particularly vulnerable to heather invasion due to the invasive plant's ability to germinate in inter-tussock spaces, its rapid vegetative growth and environmental factors such as moist infertile soils associated with these communities [21]. Another study [16] also reported displacement of native vegetation in heather-invaded sites on the Central Plateau and the author found that specialised phytophagous arthropods were negatively affected by the invasion, possibly due to a reduction in food availability, habitat loss and increased abundance of predators. The response of arthropod communities to broom invasion on the Central Plateau is not well documented. However, studies in other parts of New Zealand report an increase in generalist phytophages in broom-invaded areas [27, 28]. This suggests that both heather and broom could have different effects on the composition of arthropod assemblages in this area.

This study aimed to establish the effects of two invasive species (heather and broom) on arthropod assemblages on the Central Plateau, North Island, New Zealand. We used three different trapping methods (pitfall traps, flight intercept traps, and direct plant beating) [29]. Samples were collected from ten sites in summer and autumn, where invasive plants were either absent or present and occurring with different combinations of neighbouring plants. We first explored

the differences in diversity (Richness, Simpon's index and Shannon's index) and community composition at the order level for each sampling method for weed-invaded and non-invaded sites. We then used only the samples collected by the beating tray technique to explore further the effect of different plant pairings on arthropod diversity and community composition. We hypothesised that the impact of invasive species on arthropod communities would vary depending on the invader, plant combination and season. This study provides new information on arthropod community composition in the region and a better understanding of the impacts of invasive plants on arthropods that will assist conservation efforts.

6.2 Materials and methods

6.2.1 Site description

This study was conducted during summer 2017 through to autumn 2018 on the Central Plateau of the North Island, New Zealand. All of the woody plants used in the study occurred in natural and distinct combinations, creating an ideal system to characterise native – invasive plant interactions. We selected ten distinct sites where the four target woody shrub species: two natives *Dracophyllum* and mānuka and two invasives heather and broom, co-occur in all possible pairwise combinations (Appendix 4.1). Five replicates of paired plants, either conspecific or heterospecific, with similar sizes, were selected at each site as target plants.

6.2.2 Arthropod sampling method

Three pitfall traps (76 mm × 90 mm) covered with metal plates were laid between each paired plant. Three flight intercept traps (220 mm × 500 mm) were positioned randomly about 50 cm off the ground at each site. A 50% propylene glycol solution was used as a preservative in both traps and samples were collected after ten days. In addition, arthropods on all target plants were collected by beating similar portions of foliage from each plant onto a plastic tray. Arthropods caught in all traps were preserved in 70% ethanol and later identified to order. Sampling was first done in summer and repeated in autumn using the same techniques. The beating was done on the same target plants, and pitfall and flight intercept traps were positioned at the same locations during both seasons.

6.2.3 Data analysis

All statistical analyses were performed using R (version 3.6.3). Firstly, we explored the effect of the presence of the two exotic invasive plants on arthropod composition using the three different sampling techniques. This was done by arranging samples into three categories: heather present (n = 25), broom present (n = 25), and natives only (n = 20). The site where both invasives were simultaneously present was excluded from the analyses. Comparisons were made for each sampling method.

To investigate the impact of neighbouring plants on arthropod order richness, diversity and community composition, we used only data collected with the beating technique. This allowed us to identify 16 separate treatments including all possible pairings between the four plants species, with five samples for each

pair. Order richness, Shannon's and Simpson's diversity indices were calculated, and each variable compared between treatments using the Kruskal-Wallis test. The abundance of arthropods was compared between sites using a negative binomial generalised linear model with site as the predictor and arthropod groups as the response variables. The significance of predictors was assessed using the likelihood ratio test.

Variations in arthropod community composition were assessed by permutational multivariate analysis (PERMANOVA) based on Bray-Curtis distance using the "adonis" function in the vegan package [30]. When PERMANOVA results were significant, the "pairwise.adonis" function was used to conduct pairwise analyses between sites. The similarity percentage analysis (SIMPER) was then used to identify the arthropod groups that contributed to the differences between sites. Non-metric multidimensional scaling (NMDS) also with Bray-Curtis distance, was used to visualise the changes in arthropod community composition. PERMANOVA and NMDS were both performed using square root transformed data [31, 32].

6.3 Results

6.3.1 Arthropods at weed-invaded versus non-invaded sites

In summer, we found 13,465 arthropods belonging to 11 orders (Table 1). Order richness (R) for arthropods caught by the beating tray technique was significantly different between the sites where exotic weeds were present or absent (Kruskal-Wallis; $X^2 = 24.90$, $P < 0.001$). However, order richness

was not significantly different for arthropods caught either by flight intercept traps (Kruskal-Wallis; X^2 = 4.81, P = 0.090) or pitfall traps (Kruskal-Wallis; X^2 = 4.81, P = 0.090, Appendix 4.2). Shannon's and Simpson's diversity indices did not differ between the sites for arthropods caught by the beating tray technique (Shannon's H: X^2 = 0.09, P = 0.954 and Simpson's D: X^2 = 5.98, P = 0.050), flight intercept (Shannon's H: X^2 = 0.45, P = 0.799 and Simpson's D: X^2 = 2.85, P = 0.241) or pitfall traps (Shannon's H: X^2 = 5.60, P = 0.061 and Simpson's D: X^2 = 2.88, P = 0.237, Appendix 4.2).

In autumn, we found lower numbers of arthropods, with 6,010 total individuals belonging to 11 orders caught in this season (Table 2). Again, order richness for arthropods collected by the beating method differed significantly between the sites (Kruskal-Wallis; X^2 = 18.80, P < 0.001), while that from the flight intercept (Kruskal-Wallis; X^2 = 4.89, P = 0.087) and pitfall traps (Kruskal-Wallis; X^2 = 2.46, P = 0.293, Appendix 4.2) did not. Unlike summer, the Shannon's and Simpson's diversity indices differed significantly between the weed-invaded and non-invaded sites for the beating tray method (Shannon's H: X^2 = 12.82, P = 0.002 and Simpson's D: X^2 = 8.38, P = 0.015) but not for the flight intercept (Shannon's H: X^2 = 2.69, P = 0.260 and Simpson's D: X^2 = 0575, P = 0.750) or pitfall traps (Shannon's H: X^2 = 0.001, P = 0.999 and Simpson's D: X^2 = 0.149, P = 0.928, Appendix 4.2).

Table 1. The most abundant arthropod orders found at weed-invaded and native dominant sites in summer. Comparisons between sites were performed using a negative binomial generalised linear model. Site was the predictor, while arthropod groups were used as the response variables. The likelihood ratio test was used to assess the significance of the predictors, and significant values (P < 0.005) are highlighted in bold.

	Abundance (mean ± SE)										
Site per trap	**Collembola**	**Araneae**	**Hemiptera**	**Coleoptera**	**Hymenoptera**	**Diptera**	**Thysanoptera**	**Lepidoptera**	**Acariformes**	**Orthoptera**	**Opiliones**
Beating tray											
Broom present	0.64 ± 0.45	3.40 ± 0.50	121.12 ± 33.28	59.12 ± 16.86	1.48 ± 0.28	0.92 ± 0.32	33.92 ± 10.89	0.60 ± 0.21	26.24 ± 6.60	ND	ND
Heather present	1.76 ± 0.59	1.60 ± 0.37	0.52 ± 0.23	0.92 ± 0.24	0.28 ± 0.14	0.08 ± 0.06	7.16 ± 2.59	0.36 ± 0.16	7.20 ± 2.01	ND	ND
Natives	1.40 ± 0.57	1.50 ± 0.30	1.20 ± 0.58	9.40 ± 3.14	1.45 ± 0.43	0.10 ± 0.07	0.20 ± 0.09	0.35 ± 0.17	4.05 ± 1.25	ND	ND
X^2	2.59	11.18	58.12	50.23	17.36	15.01	29.52	1.12	17.71	-	-
P-value	0.274	**0.004**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	0.571	**< 0.001**	-	-
Flight intercept											
Broom present	0.44 ± 0.24	1.33 ± 0.62	12.11 ± 2.28	18.33 ± 3.68	1.78 ± 0.40	18.33 ± 3.93	0.33 ± 0.17	1.67 ± 0.37	10.00 ± 3.13	0.22 ± 0.15	ND
Heather present	1.67 ± 0.50	3.89 ± 0.98	0.78 ± 0.22	5.78 ± 0.85	1.22 ± 0.28	14.11 ± 3.36	45.89 ± 20.91	0.89 ± 0.35	0.33 ± 0.24	0.67 ± 0.29	ND
Natives	0.44 ± 0.18	3.00 ± 0.62	1.22 ± 0.66	8.00 ± 2.38	1.44 ± 0.53	7.11 ± 1.15	0.44 ± 0.34	1.78 ± 0.46	1.00 ± 0.29	0.67 ± 0.55	ND
X^2	7.48	7.04	28.46	14.88	0.94	8.86	27.74	3.17	20.58	1.41	-
P-value	**0.024**	**0.030**	**< 0.001**	**0.001**	0.624	**0.012**	**< 0.001**	0.205	**< 0.001**	0.494	-
Pitfall											
Broom present	3.67 ± 1.25	4.67 ± 0.62	0.33 ± 0.24	7.22 ± 1.41	6.56 ± 4.01	6.56 ± 1.19	0.44 ± 0.29	ND	20.33 ± 9.66	0.11 ± 0.11	0.44 ± 0.24
Heather present	8.44 ± 1.92	3.00 ± 0.67	0.11 ± 0.11	4.00 ± 2.51	15.22 ± 5.00	3.00 ± 2.64	ND	0.11 ± 0.11	2.22 ± 0.86	0.11 ± 0.11	0.78 ± 0.36
Natives	14.56 ±4.15	3.33 ± 0.99	0.44 ± 0.18	0.78 ± 0.78	13.22 ± 4.97	6.33 ± 1.54	ND	0.11 ± 0.11	1.00 ± 0.44	0.33 ± 0.17	0.56 ± 0.24
X^2	8.57	2.257	1.99	6.78	2.35	2.35	3.27	1.62	15.54	1.48	0.73
P-value	**0.014**	0.277	0.370	**0.034**	0.309	0.308	0.195	0.444	**< 0.001**	0.476	0.694

Table 2. The most abundant arthropod orders found at weed-invaded and native dominant sites in autumn. Comparisons between sites were performed using a negative binomial generalised linear model. Site was the predictor, while arthropod groups were used as the response variables. The likelihood ratio test was used to assess the significance of the predictors, and significant values (P < 0.005) are highlighted in bold.

	Abundance (mean ± SE)										
Site per trap	**Collembola**	**Araneae**	**Hemiptera**	**Coleoptera**	**Hymenoptera**	**Diptera**	**Thysanoptera**	**Lepidoptera**	**Acariformes**	**Orthoptera**	**Opiliones**
Beating tray											
Broom present	7.12 ± 3.09	5.56 ± 1.00	14.84 ± 5.30	9.40 ± 3.99	0.36 ± 0.13	0.76 ± 0.33	5.40 ± 3.24	0.40 ± 0.20	53.32 ± 17.84	ND	ND
Heather present	10.76 ± 3.13	3.24 ± 1.09	4.44 ± 2.01	0.36 ± 0.11	0.08 ± 0.06	0.56 ± 0.15	0.32 ± 0.17	0.32 ± 0.17	1.04 ± 0.52	ND	ND
Natives	2.95 ± 0.70	2.70 ± 0.45	1.85 ± 0.60	0.70 ± 0.24	0.15 ± 0.11	0.85 ± 0.28	0.15 ± 0.08	ND	2.15 ± 0.70	ND	ND
X^2	5.93	6.49	14.58	33.77	4.20	0.72	15.06	6.71	51.60	-	-
P-value	0.052	**0.039**	**0.001**	**< 0.001**	0.122	0.697	**0.001**	**0.035**	**< 0.001**	-	-
Flight intercept											
Broom present	33.33 ±11.83	33.33 ± 9.99	9.52 ± 5.32	34.98 ± 10.26	33.33 ± 8.82	40.63 ± 10.03	ND	16.67 ± 11.79	46.30 ± 14.90	11.11 ± 6.05	11.11 ± 11.11
Heather present	4.04 ± 2.20	12.50 ± 3.61	7.94 ± 5.38	5.35 ± 2.40	40.00 ± 10.00	35.56 ± 6.43	11.11 ± 11.11	11.11 ± 7.35	7.41 ± 7.41	44.44 ± 12.34	11.11 ± 11.11
Natives	25.25 ±11.11	12.50 ± 3.61	12.70 ± 11.03	7.41 ± 0.87	35.56 ± 11.44	31.75 ± 9.12	11.11 ± 11.11	27.78 ± 21.11	1.85 ± 1.85	19.44 ± 8.10	ND
X^2	7.77	7.12	0.167	18.10	0.24	0.59	1.62	1.36	48.34	6.56	1.62
P-value	**0.021**	**0.029**	0.920	**< 0.001**	0.885	0.745	0.444	0.505	**< 0.001**	**0.038**	0.444
Pitfall											
Broom present	5.78 ± 0.76	1.00 ± 0.33	0.22 ± 0.15	2.11 ± 0.75	1.89 ± 0.73	2.44 ± 1.51	ND	0.11 ± 0.11	8.33 ± 3.02	0.11 ± 0.11	0.89 ± 0.45
Heather present	19.89 ± 8.48	1.44 ± 0.63	0.11 ± 0.11	1.11 ± 0.59	4.22 ± 1.64	3.22 ± 1.52	ND	ND	3.78 ± 0.85	0.33 ± 0.24	1.33 ± 0.58
Natives	21.11 ± 5.00	1.33 ± 0.44	0.22 ± 0.22	1.22 ± 0.32	6.56 ± 2.67	7.22 ± 1.61	ND	ND	9.22 ± 6.03	0.11 ± 0.11	0.89 ± 0.45
X^2	10.72	0.52	0.35	1.63	4.09	3.39	-	2.20	2.67	1.19	6.08
P-value	**0.004**	0.772	0.840	0.442	0.129	0.184	-	0.333	0.263	0.550	**0.048**

A PERMANOVA revealed that arthropod composition (relative abundance of insects belonging to each order) differed significantly between weed-invaded and non-invaded sites for all trapping methods in both seasons (Table 3, Figure 1).

Table 3. Statistical results for differences in community composition between treatments (broom present, heather present and natives) after PERMANOVA, for three trapping methods.

	Summer		**Autumn**	
Trapping method	**Pseudo-*F***	***P***	**Pseudo-*F***	***P***
Beating technique	$F_{2,67} = 8.49$	< 0.001	$F_{2,67} = 6.70$	< 0.001
Flight intercept	$F_{2,24} = 7.75$	< 0.001	$F_{2,67} = 3.06$	0.003
Pitfall traps	$F_{2,24} = 4.53$	< 0.001	$F_{2,67} = 2.12$	0.036

The similarity percentage analyses revealed that different arthropod orders contributed to these differences depending on the season and trapping method used (Figure 1). The beating traps showed a high overlap among treatments in both summer and autumn. However, in flight intercept and pitfall traps, a higher separation between treatments was evident, with little overlap between the samples collected in heather-invaded and broom-dominated sites (Figure 1).

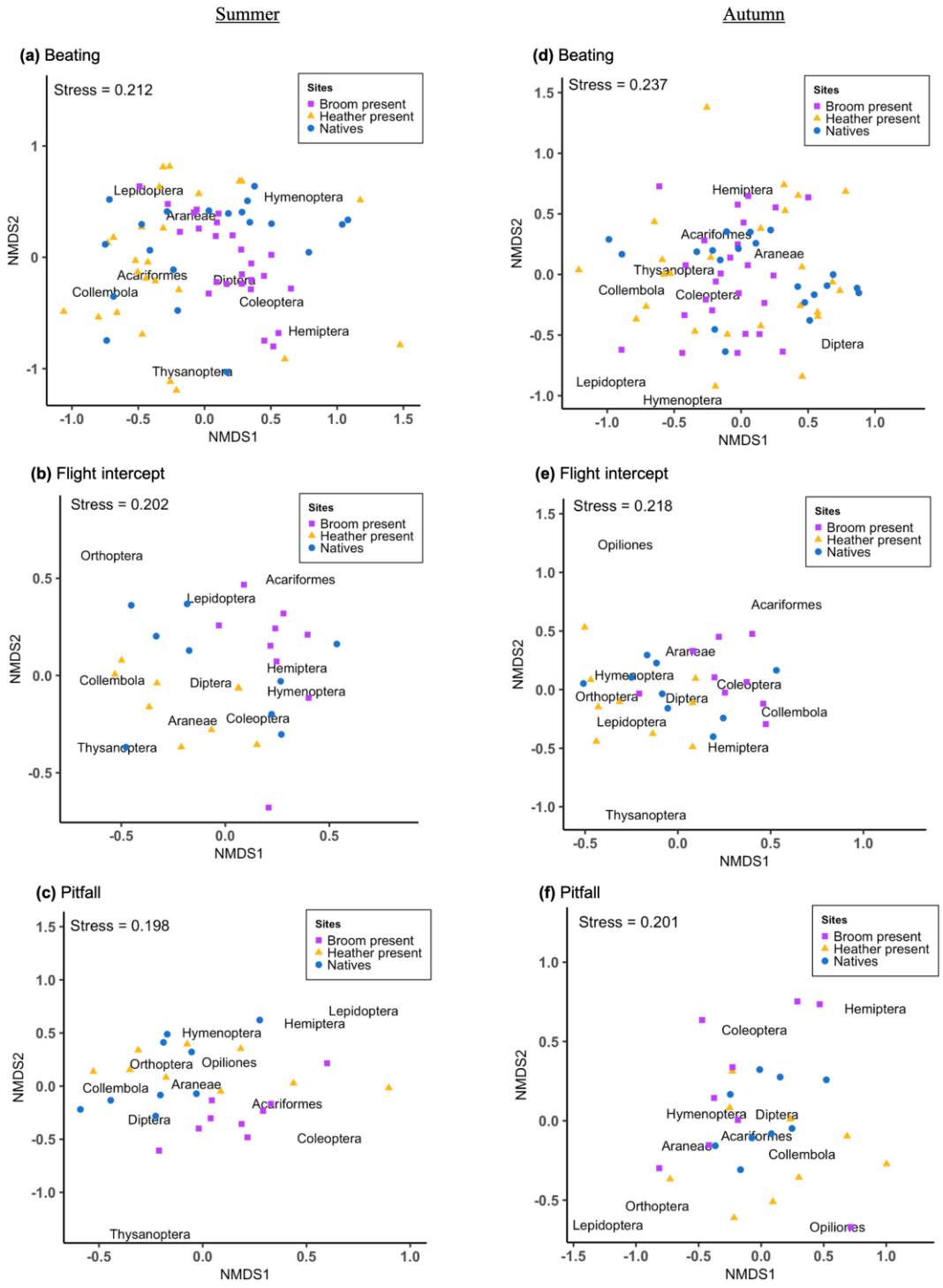

Figure 1. NMDS plot for arthropod community composition in weed-invaded and non-invaded sites in summer (a), (b), (c) and autumn (d), (e), (f). Arthropods were caught by beating tray technique, flight intercept and pitfall traps.

6.3.2 Arthropods present on target plants under different plant species combinations

With the aim to explore the effect of different plant species combinations on arthropod communities directly interacting with our target plants, we only analysed the samples collected by beating the foliage of each target plant (heather, broom, mānuka or *Dracophyllum*) and classified samples according to the predominant plant combination present at each site (e.g., broom with mānuka, broom with *Dracophylum*, broom with heather, broom with conspecifics, and so forth). Likelihood ratio tests revealed different orders to be affected differently depending on the target plant and season (Table 4, Table 5).

The Simpson diversity index was not affected by the neighbouring plant composition in all cases, except for broom in summer. With broom as the target plant, the Shanon diversity index was significantly different between sites where this plant co-occurred with either conspecifics or heterospecifics in both seasons. The Shanon diversity index for arthropods on mānuka only differed significantly between the sites in autumn. Order richness (R) on plants was found to differ for all target plant combinations in at least one of the two seasons (Table 6).

The permutational multivariate analysis of variance (PERMANOVA) revealed significant differences in arthropod order-level community composition on all target plants under different plant species combination (i.e. target plants paired with either conspecifics or one of the three heterospecific plants) during both seasons, except for broom in autumn (Table 7).

Table 4. Arthropods on target plants when paired with either conspecifics or heterospecific neighbours in summer. Arthropods were caught by beating a similar proportion of foliage of each target plant on a tray. Comparisons between sites were performed using a negative binomial generalised linear model. Site was used as the predictor while arthropod groups were the response variables. The likelihood ratio test was used to assess the significance of the predictors, and significant values ($P < 0.005$) are highlighted in bold. N = 5 for each treatment.

	Abundance (mean ± SE)								
	Collembola	**Araneae**	**Hemiptera**	**Coleoptera**	**Hymenoptera**	**Diptera**	**Thysanoptera**	**Lepidoptera**	**Acariformes**
Broom as target plant									
Broom - Broom	ND	1.00 ± 0.55	103.20 ± 27.74	31.60 ± 8.68	1.40 ± 1.17	0.60 ± 0.40	71.00 ± 36.51	0.60 ± 0.40	18.80 ± 9.71
Broom - Heather	0.20 ± 0.20	1.20 ± 0.97	544.20 ± 95.85	2.20 ± 0.37	1.00 ± 0.77	ND	12.40 ± 4.95	2.20 ± 1.24	6.80 ± 1.98
Broom - *Dracophyllum*	2.20 ± 2.20	3.20 ± 0.66	310.80 ± 115.01	108.20 ± 16.09	1.20 ± 0.20	2.80 ± 1.24	23.20 ± 11.93	0.20 ± 0.20	5.80 ± 3.48
Broom - Mānuka	0.60 ± 0.60	4.80 ± 1.39	184.40 ± 47.32	151.40 ± 60.39	1.60 ± 0.60	0.80 ± 0.37	75.20 ± 26.73	0.20 ± 0.20	12.00 ± 4.24
X^2	25.63	8.38	14.41	38.20	0.44	12.28	7.06	7.63	3.697
P-value	**< 0.001**	**0.039**	**0.002**	**< 0.001**	0.932	**0.006**	0.070	0.054	0.296
***Dracophyllum* as target plant**									
Dracophyllum - *Dracophyllum*	0.80 ± 0.37	0.80 ± 0.58	3.40 ± 1.99	3.80 ± 1.59	1.20 ± 0.49	0.40 ± 0.24	0.20 ± 0.20	0.20 ± 0.20	ND
Dracophyllum - Heather	5.40 ± 2.18	1.20 ± 0.58	0.20 ± 0.20	2.00 ± 0.84	0.20 ± 0.20	ND	3.60 ± 1.08	ND	20.20 ± 3.51
Dracophyllum - Mānuka	4.40 ± 1.69	1.40 ± 0.68	0.80 ± 0.80	2.80 ± 1.39	0.60 ± 0.24	ND	0.40 ± 0.24	0.80 ± 0.58	9.20 ± 3.06
Dracophyllum - Broom	0.20 ± 0.20	5.60 ± 1.03	4.80 ± 1.50	2.60 ± 0.98	2.00 ± 0.55	0.20 ± 0.20	ND	0.40 ± 0.24	62.80 ± 25.07
X^2	13.60	14.23	8.63	0.929	53.95	4.50	19.31	4.90	41.06
P-value	**0.004**	**0.003**	**0.035**	0.819	**< 0.001**	0.212	**< 0.001**	0.180	**< 0.001**
Heather as target plant									
Heather - Heather	1.00 ± 0.77	0.8 ± 0.58	1.00 ± 1.00	ND	ND	ND	21.20 ± 7.84	0.40 ± 0.40	11.60 ± 5.71

Heather - *Dracophyllum*	2.20 ± 0.49	2.00 ± 1.05	0.20 ± 0.20	0.80 ± 0.37	ND	0.20 ± 0.20	10.20 ± 7.79	ND	3.60 ± 1.21
Heather - Mānuka	0.20 ± 0.20	0.40 ± 0.24	1.20 ± 0.49	0.60 ± 0.40	0.20 ± 0.20	ND	0.40 ± 0.40	0.20 ± 0.20	ND
Heather - Broom	1.20 ± 0.58	1.60 ± 0.60	41.20 ± 12.07	0.20 ± 0.20	0.40 ± 0.40	0.60 ± 0.40	3.00 ± 1.05	0.20 ± 0.20	65.60 ± 20.08
X^2	7.45	4.90	26.19	6.46	3.29	5.81	13.91	2.29	33.54
P-value	0.059	0.179	**< 0.001**	0.091	0.349	0.121	**0.003**	0.514	**< 0.001**
Mānuka as target plant									
Mānuka - Mānuka	0.20 ± 0.20	2.20 ± 0.73	0.60 ± 0.24	10.60 ± 2.62	2.80 ± 1.36	0.20 ± 0.20	ND	0.20 ± 0.20	4.20 ± 2.85
Mānuka - Heather	ND	3.60 ± 0.87	ND	1.20 ± 0.37	1.00 ± 0.55	ND	0.40 ± 0.24	1.20 ± 0.58	0.60 ± 0.40
Mānuka - *Dracophyllum*	0.20 ± 0.20	1.60 ± 0.40	ND	20.40 ± 11.25	1.20 ± 0.80	ND	0.20 ± 0.20	0.20 ± 0.20	2.80 ± 1.07
Mānuka - Broom	0.20 ± 0.20	2.40 ± 0.51	2.40 ± 1.36	1.80 ± 0.80	1.20 ± 0.37	0.20 ± 0.20	0.20 ± 0.20	1.60 ± 0.81	31.80 ± 6.51
X^2	1.73	4.17	12.66	15.94	3.24	2.77	2.77	6.46	19.58
P-value	0.631	0.243	**0.005**	**0.001**	0.357	0.428	0.428	0.091	**< 0.001**

Table 5. Arthropods on target plants when paired with either conspecifics or heterospecific neighbours in autumn. Arthropods were caught by beating a similar proportion of foliage of each target plant on a tray. Comparisons between sites were performed using a negative binomial generalised linear model. Site was used as the predictor while arthropod groups were the response variables. The likelihood ratio test was used to assess the significance of the predictors, and significant values ($P < 0.005$) are highlighted in bold. N = 5 for each treatment.

	Abundance (mean ± SE)								
	Collembola	**Araneae**	**Hemiptera**	**Coleoptera**	**Hymenoptera**	**Diptera**	**Thysanoptera**	**Lepidoptera**	**Acariformes**
Broom as target plant									
Broom - Broom	7.60 ± 4.49	10.20 ±3.07	39.20 ± 23.58	18.00 ± 13.27	0.20 ± 0.20	0.40 ± 0.40	17.00 ± 15.78	1.00 ± 0.77	132.80 ±70.26
Broom - Heather	0.20 ± 0.20	2.20 ± 0.86	0.80 ± 0.80	ND	ND	ND	0.20 ± 0.20	ND	18.60 ± 12.99
Broom - *Dracophyllum*	1.40 ± 0.75	4.20 ± 2.99	6.40 ± 5.90	3.80 ± 1.85	ND	0.20 ± 0.20	1.20 ± 0.80	0.20 ± 0.20	15.00 ± 8.97
Broom - Mānuka	5.00 ± 3.82	4.80 ± 0.86	7.00 ± 2.83	1.00 ± 0.45	0.40 ± 0.24	0.20 ± 0.20	1.20 ± 0.80	ND	14.40 ± 6.93
X^2	7.05	6.59	8.77	14.71	4.50	2.29	6.48	6.00	11.75
P-value	0.070	0.086	**0.032**	**0.002**	0.212	0.514	0.091	0.112	**0.008**
***Dracophyllum* as target plant**									
Dracophyllum - *Dracophyllum*	6.00 ± 2.05	2.00 ± 0.63	4.00 ± 1.41	1.20 ± 0.58	0.40 ± 0.40	ND	0.40 ± 0.24	ND	4.80 ± 1.50
Dracophyllum - Heather	3.80 ± 1.50	8.40 ± 4.92	1.20 ± 0.37	0.20 ± 0.20	0.40 ± 0.24	1.00 ± 0.45	1.00 ± 0.55	ND	1.00 ± 0.63
Dracophyllum - Mānuka	2.20 ± 0.80	1.60 ± 0.68	ND	0.60 ± 0.40	ND	0.40 ± 0.24	0.20 ± 0.20	ND	0.60 ± 0.24
Dracophyllum - Broom	19.20 ± 14.07	3.60 ± 1.60	9.60 ± 6.49	18.00 ± 15.06	0.80 ± 0.37	0.80 ± 0.37	7.60 ± 3.70	ND	81.00 ± 39.91
X^2	9.25	6.90	17.17	15.18	5.42	7.70	13.79	-	31.42
P-value	**0.026**	0.075	**0.001**	**0.002**	0.144	0.053	**0.003**	-	**< 0.001**

Heather as target plant									
Heather - Heather	29.80 ± 8.90	1.40 ± 0.40	1.20 ± 0.97	0.60 ± 0.24	ND	0.80 ± 0.37	ND	1.40 ± 0.68	1.00 ± 1.00
Heather - *Dracophyllum*	18.60 ± 6.61	1.60 ± 0.60	1.40 ± 0.75	0.60 ± 0.40	ND	ND	ND	0.20 ± 0.20	3.00 ± 2.28
Heather - Mānuka	1.60 ± 1.12	1.40 ± 068	0.20 ± 0.20	0.20 ± 0.20	ND	0.80 ± 0.37	0.60 ± 0.60	ND	0.20 ± 0.20
Heather - Broom	0.40 ± 0.40	2.40 ± 1.44	1.00 ± 1.00	2.00 ± 1.76	ND	0.20 ± 0.20	ND	ND	20.80 ± 9.74
X^2	17.59	1.21	1.97	3.80	-	7.58	0.28	10.03	12.47
P-value	**0.001**	0.750	0.579	0.284	-	0.055	0.963	**0.018**	**0.006**
Mānuka as target plant									
Mānuka - Mānuka	1.80 ± 0.86	3.80 ± 0.46	1.20 ± 0.73	0.60 ± 0.60	0.20 ± 0.20	1.20 ± 0.58	ND	ND	2.00 ± 2.00
Mānuka - Heather	ND	3.40 ± 1.29	18.20 ± 7.72	0.20 ± 020	ND	0.20 ± 0.20	ND	ND	ND
Mānuka - *Dracophyllum*	1.80 ± 0.80	3.40 ± 1.36	2.20 ± 1.50	0.40 ± 0.40	ND	1.80 ± 0.80	ND	ND	1.20 ± 0.73
Mānuka - Broom	2.40 ± 1.29	5.00 ± 1.10	12.00 ± 3.15	6.20 ± 2.48	0.40 ± 0.40	2.20 ± 1.50	ND	0.80 ± 0.58	23.40 ± 12.44
X^2	9.84	1.35	15.03	9.47	3.29	4.89	-	6.43	15.38
P-value	**0.020**	0.717	**0.002**	**0.024**	0.349	0.180	-	0.093	**0.002**

Table 6. Richness, Shannon's and Simpson's diversity indices for arthropods (at order level) on each target plant paired with conspecific and heterospecific neighbours in summer and autumn. P-values calculated using the Kruskal-Wallis test and significant values (P < 0.005) are highlighted in bold. N = 5.

Target plants	Sites (mean ± SE)				Differences X^2	DF	*P*
Summer							
Broom	**BB**	**BH**	**BD**	**BM**			
Richness	5.80 ± 0.66	5.60 ± 0.24	6.80 ± 0.58	6.60 ± 0.51	3.52	3	0.319
Shannon	1.20 ± 0.15	0.26 ± 0.08	0.90 ± 0.14	1.09 ± 0.10	9.89	3	**0.020**
Simpson	0.55 ± 0.09	0.11 ± 0.04	0.47 ± 0.07	0.57 ± 0.05	10.79	3	**0.013**
Heather	**HB**	**HH**	**HD**	**HM**			
Richness	5.60 ± 0.24	3.20 ± 058	4.40 ± 0.87	2.40 ± 0.40	9.84	3	**0.020**
Shannon	1.01 ± 0.12	0.66 ± 0.19	1.11 ± 0.17	0.77 ± 0.21	2.11	3	0.549
Simpson	0.58 ± 0.04	0.37 ± 0.11	0.65 ± 0.07	0.73 ± 0.19	6.14	3	0.105
Dracophyllum	**DB**	**DH**	**DD**	**DM**			
Richness	5.40 ± 0.24	4.60 ± 0.04	3.20 ± 0.20	5.00 ± 0.89	8.27	3	**0.041**
Shannon	0.86 ± 0.13	1.05 ± 0.04	0.98 ± 0.06	1.25 ± 0.21	2.22	3	0.528
Simpson	0.42 ± 0.07	0.57 ± 0.03	0.68 ± 0.08	0.67 ± 0.08	3.02	3	0.061
Mānuka	**MB**	**MH**	**MD**	**MM**			
Richness	5.20 ± 0.58	3.80 ± 0.58	3.40 ± 0.51	4.40 ± 0.75	4.19	3	0.241
Shannon	0.89 ± 0.22	1.24 ± 0.13	0.76 ± 0.18	0.93 ± 0.19	3.64	3	0.303
Simpson	0.43 ± 0.10	0.77 ± 0.05	0.48 ± 0.14	0.50 ± 0.09	5.90	3	0.116
Autumn							
Broom	**BB**	**BH**	**BD**	**BM**			
Richness	5.60 ± 0.68	2.20 ± 0.37	4.40 ± 0.51	5.00 ± 0.89	9.41	3	**0.024**
Shannon	1.16 ± 0.18	0.49 ± 0.18	1.23 ± 0.08	1.05 ± 0.13	7.98	3	**0.046**
Simpson	0.58 ± 0.09	0.37 ± 0.14	0.75 ± 0.07	0.57 ± 0.04	7.42	3	0.060
Heather	**HB**	**HH**	**HD**	**HM**			
Richness	2.80 ± 0.66	4.00 ± 0.55	3.80 ± 0.37	2.80 ± 0.49	4.17	3	0.243
Shannon	0.64 ± 0.16	0.66 ± 0.21	0.71 ± 0.19	0.81 ± 0.22	0.65	3	0.884
Simpson	0.50 ± 0.16	0.34 ± 0.13	0.39 ± 0.13	0.79 ± 0.10	5.15	3	0.161
Dracophyllum	**DB**	**DH**	**DD**	**DM**			
Richness	6.40 ± 0.81	4.60 ± 0.81	5.20 ± 0.49	3.20 ± 0.37	9.07	3	0.028
Shannon	1.12 ± 0.08	1.16 ± 0.18	1.42 ± 0.11	1.03 ± 0.11	4.81	3	0.186
Simpson	0.57 ± 0.04	0.75 ± 0.09	0.77 ± 0.04	0.83 ± 0.08	5.74	3	0.125
Mānuka	**MB**	**MH**	**MD**	**MM**			
Richness	5.60 ± 0.24	2.20 ± 0.37	3.40 ± 0.68	3.40 ± 0.24	12.31	3	**0.006**
Shannon	1.35 ± 0.11	0.56 ± 0.18	1.01 ± 0.18	1.08 ± 0.07	10.03	3	**0.018**
Simpson	0.68 ± 0.06	0.39 ± 0.13	0.68 ± 0.04	0.69 ± 0.04	6.61	3	0.085

Abbreviations: Broom (B), Heather (H), *Dracophyllum* (D) and Mānuka (M). Combinations of abbreviations illustrate plant pairs. E.g. BB = broom paired with broom and BH = broom with heather

Table 7. PERMANOVA results for differences in arthropod community composition on target plants at sites where they either occur with conspecifics or one of the three heterospecific plants. N = 5 for each treatment.

	Summer		Autumn	
Target plant	**Pseudo-*F***	***P***	**Pseudo-*F***	***P***
Broom	$F_{3,16} = 5.12$	< 0.001	$F_{3,16} = 1.54$	0.098
Heather	$F_{3,16} = 6.12$	< 0.001	$F_{3,16} = 3.35$	0.002
Dracophyllum	$F_{3,16} = 4.30$	< 0.001	$F_{3,16} = 2.90$	0.002
Mānuka	$F_{3,16} = 3.71$	0.002	$F_{3,16} = 3.38$	0.002

A NMDS plot of the community composition (Figure 2), shows that in the case of heather and broom, the arthropod composition shows little overlap when plants are paired with conspecifics versus when paired with another invasive both in summer and autumn. A similar trend was observed for mānuka when paired with conspecifics versus either of the invasive plants, but this trend was not observed for *Dracophyllum*. Consistent with these observations, pairwise comparisons showed significant differences between treatments sharing the same target plant in both seasons, with very few exceptions (Appendix 4.3).

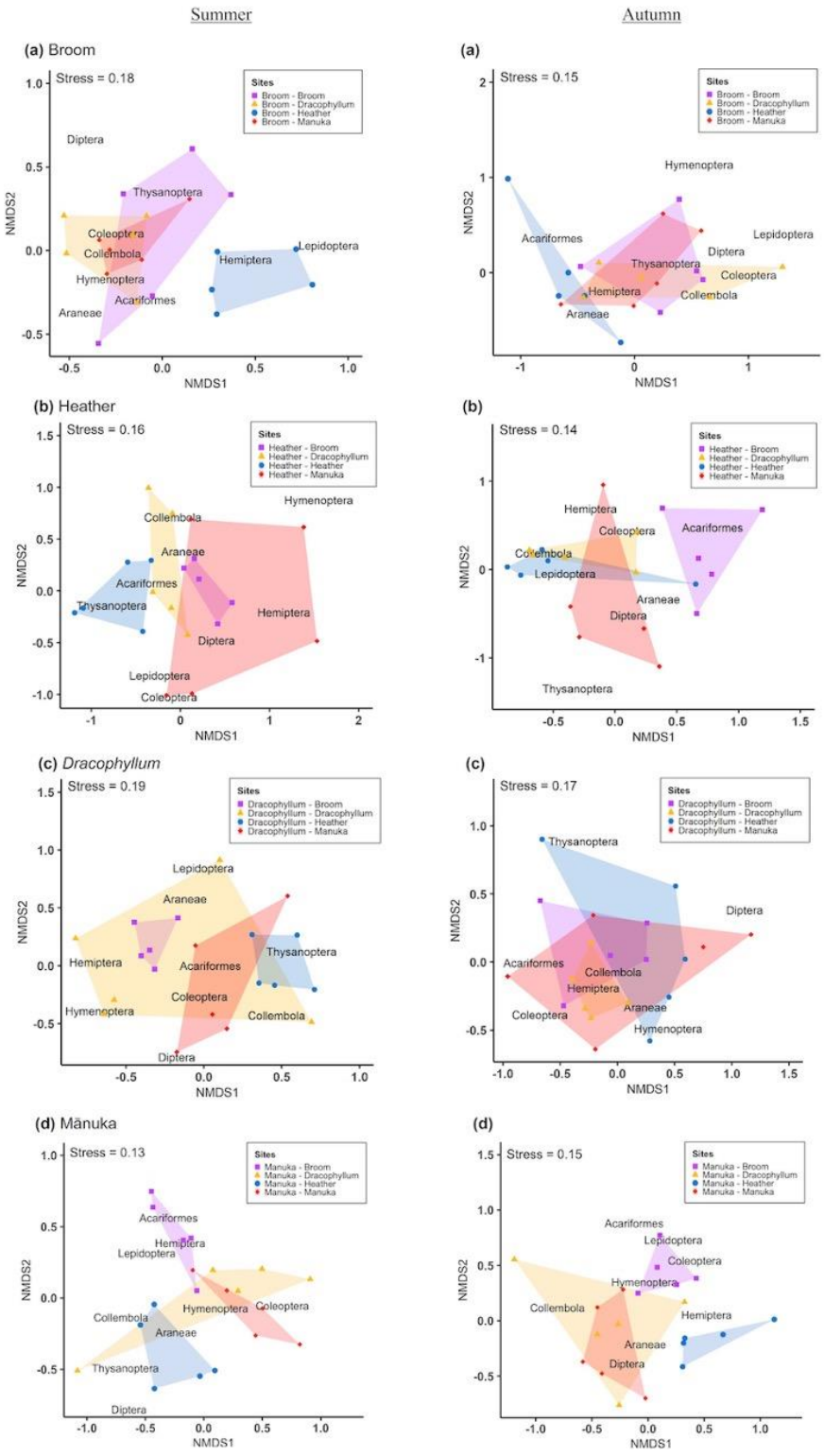

Figure 2. NMDS plots showing the arthropod community composition on (a) broom (b) heather (c) *Dracophyllum* and (d) mānuka paired with different plants in summer and autumn. Arthropods collected by the beating tray technique. N = 5 for each group.

6.4 Discussion

Exotic plant invasion modifies vegetation structure and leads to a shift in plant species composition in the new habitat [33]. This may be detrimental for other community members like arthropods that rely on surrounding native vegetation for food, shelter and reproduction sites. Here, we demonstrate that during plant invasion, arthropod assemblages are affected variably by the invading species. Broom typically increased arthropod abundance while heather is associated with a reduction in arthropod abundance. Also, arthropod-plant associations can be affected by the identity of the neighbouring plants. These effects can be season dependent.

Our results only partially support the often-reported observation that arthropod abundance and diversity are decreased in habitats dominated by exotic weeds [15], but rather indicate that the responses of arthropod communities depend on the identity of the invasive plant. While invasive plants may indeed reduce the resources for specialist herbivores, arthropod groups occupying other, more generalist, ecological niches may thrive in these environments [15].

In this study, we found a high number of Acariformes (mostly detritivore oribatid mites) and Coleoptera (mostly silken fungus beetles) associated with broom in pitfall traps, while only small numbers of these groups were associated with heather. This is an example of how different invasive plants can provide different resources. Here decaying vegetation is creating optimal habitats for detritivores and fungivores [34, 35].

In the flight intercept traps Hemiptera, Coleoptera, Araneae, Acariformes and Diptera were significantly more abundant in broom-infested sites in summer and autumn. Meanwhile, heather had less pronounced effects with only a substantial increase in Thysanoptera (thrips) and Araneae in summer and Orthoptera during autumn. Thrips are well known generalist florivores and have been suggested to contribute to heather pollination in other ecosystems [36]. Many invasives have large floral displays or many flowers that can attract generalist pollinators and florivores [37]. It is therefore reasonable to assume that heather attracts native florivores to aid in its reproduction and dispersal, but no hard evidence was found that Hymenoptera (in particular native pollinators) captured by this method were significantly impacted by the presence of heather or broom.

The high number of Hemiptera found at the broom-invaded sites, were predominantly exotic broom psyllids that were introduced from England to control the spread of broom in New Zealand [38]. In comparison, the abundance of Coleoptera and Orthoptera were predominantly native generalist herbivores that would have been using the invasive species as a food source or habitat to live in. Araneae and some Diptera are predators or parasitoids of herbivores, thus their increase may be explained by higher abundance of their prey species [39]. Certain plant architectures, such as the intricate and dense branching pattern of heather, can also create suitable habitats for spiders and other predators [16, 40].

The composition of arthropods collected by the beating tray technique showed a similar trend as that of flight intercept traps at broom-invaded sites but with higher abundance of Hemiptera, Coleoptera, Thysanoptera and Acariformes. Contrary, a reduction in abundance of some arthropod groups (Coleoptera, Diptera, Thysanoptera and Orthorptera) was observed at the heather invaded-sites for samples collected by the beating tray technique compared with flight intercept traps. It is relevant to note that many Coleoptera caught by beating the foliage of mānuka were mānuka beetles, which are endemic insects typically associated with this plant. However, mānuka beetles have been reported to feed on other plants, and they are considered to be pasture pests in some regions [27], supporting our previous observation that native insects feed on both native and exotic invasive plants [41].

An earlier study assessing the impact of invasion by heather on native invertebrates on the Central Plateau also showed some variation in invertebrate assemblages [16]. Consistent with our findings, the author found that invasion by heather was usually associated with fewer plant-eaters, high abundance of thrips (pollen eaters) and increased predators [16]. A comparative study of the arthropods associated with broom in two native (France and Scotland) and non-native (New Zealand and Australia) ranges [28], using the beating tray method, found generalist phytophages to be dominant on broom in exotic habitats and specialists dominant on broom in the native habitats. Thus, the overall abundance of arthropods

was high but not significantly different between the two habitats. We also found high arthropod abundance in broom-invaded sites for several groups, suggesting that not only generalist herbivores, but arthropods occupying other niches benefit from the resources provided by this invasive species. Generally, mechanisms promoting such facilitative interaction between invasive plants and arthropods include habitat modification, diversification of food source and availability of exploitable hosts [42].

Plant species composition can be used as a predictor of arthropod assemblages, as revealed by a study using multiple sites with different vegetation covers and a range of sampling methods [43]. However, that study reveals that this is not necessarily due to the direct use of particular plants as a resource, but their correlation with some other factors (i.e., microclimate, habitat structure, changes in trophic webs). Our results strongly support the hypothesis that it is plant community composition, rather than the presence of invaders only, that is a strong driver of change in arthropod assemblages. We found significant differences in arthropod community composition between sites where the same invasive was present but in combination with other species, and the same was true for natives.

Overall, our results highlight that it is difficult to generalise when considering the impacts of invasive plants on arthropod communities, and that sampling multiple sites with different plant assemblages, using a variety of different trapping methods over multiple seasons are needed to

elucidate the complex effects of invasive plants on arthropod communities and their associated ecosystem services. Further studies investigating lower taxonomic levels, focusing on native and endemic arthropods, and with a multitrophic approach [44] will be of great assistance to expand these findings and support conservation efforts.

6.5 Conclusion

Here, we assessed the arthropod community at sites where exotic invasive weeds were present or absent and investigated the arthropods' abundant on target plants during an invasion. Our work demonstrates that arthropod community composition in response to plant invasion is dependent on the identity of the invasive species and the composition of the nearby vegetation and shows that while some exotic invasive plants may reduce arthropod abundance and diversity in the new habitat, others may have the opposite effect. We also show that some of these impacts may be seasonal. This work emphasises the need for incorporating plant community composition, seasonality, and diverse sampling methods in future studies.

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6.8 Appendix 4: Supplementary information

Appendix 4.1

Table A1. Coordinates and plant species composition at study sites.

Site	Coordinates	Dominant plants	Description
1	Long. 175.685483 – Lat. -39.432933	Mānuka	Natives
2	Long. 175.685483 – Lat. -39.432933	Mānuka and *Dracophyllum*	Natives
3	Long. 175.68785 – Lat. - 39.4206	Heather and *Dracophyllum*	Heather present
4	Long. 175.73705 – Lat. -39.311217	*Dracophyllum*	Natives
5	Long. 175.734317 – Lat. -39.314683	Heather and Mānuka	Heather present
6	Long. 175.6888 – Lat. -39.415467	Heather	Heather present
7	Long. 175.737467 – Lat. -39.315117	Broom	Broom present
8	Long. 175.737 – Lat. -39.315383	Broom and Mānuka	Broom present
9	Long. 175.732783 – Lat. -39.3142	Broom and *Dracophyllum*	Broom present
10	Long. 175.3907 – Lat. -39.244033	Heather and Broom	Heather and broom present

Appendix 4.2

Table A2. Order richness (R) and diversity indices for arthropods collected from sites where exotic invasive plants heather and broom were either present or absent.

Trap	Site (mean ± SE)		
	Broom present	**Heather present**	**Natives**
Summer			
Beating			
Order richness (R)	5.96 ± 0.26	3.72 ± 0.29	4.00 ± 0.34
Shannon (H)	0.95 ± 0.07	0.97 ± 0.08	0.98 ± 0.09
Simpson (D)	0.49 ± 0.04	0.62 ± 0.05	0.58 ± 0.05
Flight intercept			
Order richness (R)	7.00 ± 0.47	7.33 ± 0.33	6.22 ± 0.32
Shannon (H)	1.45 ± 0.10	1.42 ± 0.11	1.56 ± 0.07
Simpson (D)	0.70 ± 0.05	0.68 ± 0.06	0.78 ± 0.03
Pitfall			
Order richness (R)	6.44 ± 0.24	5.22 ± 0.49	5.67 ± 0.41
Shannon (H)	1.51 ± 0.67	1.22 ± 0.12	1.25 ± 0.09
Simpson (D)	0.74 ± 0.03	0.64 ± 0.05	0.65 ± 0.04
Autumn			
Beating			
Order richness (R)	5.40 ± 0.31	3.48 ± 0.28	3.80 ± 0.29
Shannon (H)	1.18 ± 0.05	0.77 ± 0.90	1.14 ± 0.07
Simpson (D)	0.63 ± 0.03	0.53 ± 0.06	0.74 ± 0.03
Flight intercept			
Order richness (R)	6.22 ± 0.40	4.78 ± 0.40	5.22 ± 0.55
Shannon (H)	1.41 ± 0.10	1.15 ± 0.10	1.27 ± 0.08
Simpson (D)	0.70 ± 0.04	0.62 ± 0.06	0.67 ± 0.04
Pitfall			
Order richness (R)	4.78 ± 0.40	5.11 ± 0.35	5.67 ± 0.33
Shannon (H)	1.18 ± 0.10	1.16 ± 0.15	1.21 ± 0.09
Simpson (D)	0.65 ± 0.04	0.59 ± 0.08	0.62 ± 0.05

Appendix 4.3

Table A3. Pairwise comparisons for arthropod community composition on target plants paired with conspecifics and heterospecific neighbours for both summer and autumn. Pairwise performed using the "pairwise.adonis" function in R. Significant values (P<0.005) are highlighted in bold.

		Test of differences			
Sites/plant pairs	**DF**	**Sum of Squares**	***F*-value**	**R^2**	***P*-value**
Summer					
Broom as target plant					
Broom - Broom Vs. Broom - Heather	1	0.368	7.818	0.494	**0.007**
Broom - Broom Vs. Broom - *Dracophyllum*	1	0.163	3.017	0.274	**0.016**
Broom - Broom Vs. Broom - Mānuka	1	0.071	1.381	0.147	0.273
Broom - Heather Vs. Broom - *Dracophyllum*	1	0.306	9.154	0.534	**0.007**
Broom - Heather Vs. Broom - Mānuka	1	0.355	11.344	0.586	**0.007**
Broom - *Dracophyllum* Vs. Broom - Mānuka	1	0.048	1.250	0.135	0.310
***Dracophyllum* as target plant**					
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Heather	1	0.866	5.947	0.426	**0.009**
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Mānuka	1	0.537	2.865	0.264	**0.028**
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Broom	1	0.659	4.558	0.363	**0.010**
Dracophyllum - Heather Vs. *Dracophyllum* - Mānuka	1	0.109	1.230	0.133	0.304
Dracophyllum - Heather Vs. *Dracophyllum* - Broom	1	0.446	9.850	0.552	**0.008**
Dracophyllum - Mānuka Vs. *Dracophyllum* - Broom	1	0.389	4.468	0.358	**0.016**
Heather as target plant					
Heather - Heather Vs. Heather - *Dracophyllum*	1	0.330	2.878	0.265	**0.032**
Heather - Heather Vs. Heather - Mānuka	1	1.226	6.597	0.452	**0.006**
Heather - Heather Vs. Heather - Broom	1	0.595	7.221	0.474	**0.012**
Heather - *Dracophyllum* Vs. Heather - Mānuka	1	0.978	5.282	0.398	**0.008**
Heather - *Dracophyllum* Vs. Heather - Broom	1	0.712	8.728	0.522	**0.010**
Heather - *Mānuka* Vs. Heather - Broom	1	1.067	6.983	0.466	**0.009**
Mānuka as target plant					
Mānuka - Mānuka Vs. Mānuka - Heather	1	0.395	4.239	0.346	**0.022**

Mānuka - Mānuka Vs. Mānuka - *Dracophyllum*	1	0.135	0.991	0.110	0.461
Mānuka - Mānuka Vs. Mānuka - Broom	1	0.499	6.694	0.456	**0.015**
Mānuka - Heather Vs. Mānuka - *Dracophyllum*	1	0.324	2.276	0.221	**0.081**
Mānuka - Heather Vs. Mānuka - Broom	1	0.565	6.998	0.467	**0.008**
Mānuka - *Dracophyllum* Vs. Mānuka - Broom	1	0.495	3.997	0.333	**0.010**
Autumn					
Dracophyllum as target plant					
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Heather	1	0.215	1.802	0.184	0.102
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Mānuka	1	0.348	3.037	0.275	**0.019**
Dracophyllum - *Dracophyllum* Vs. *Dracophyllum* - Broom	1	0.331	3.689	0.316	**0.023**
Dracophyllum - Heather Vs. *Dracophyllum* - Mānuka	1	0.258	1.405	0.149	0.302
Dracophyllum - Heather Vs. *Dracophyllum* - Broom	1	0.469	2.948	0.269	**0.007**
Dracophyllum - Mānuka Vs. *Dracophyllum* - Broom	1	0.758	4.917	0.381	**0.008**
Heather as target plant					
Heather - Heather Vs. Heather - *Dracophyllum*	1	0.152	1.252	0.135	0.269
Heather - Heather Vs. Heather - Mānuka	1	0.460	2.346	0.227	**0.055**
Heather - Heather Vs. Heather - Broom	1	0.977	6.586	0.451	**0.027**
Heather - *Dracophyllum* Vs. Heather - Mānuka	1	0.381	2.138	0.211	**0.060**
Heather - *Dracophyllum* Vs. Heather - Broom	1	0.699	5.353	0.401	**0.025**
Heather - *Mānuka* Vs. Heather - Broom	1	0.612	2.989	0.272	**0.038**
Mānuka as target plant					
Mānuka - Mānuka Vs. Mānuka - Heather	1	0.590	5.364	0.401	**0.011**
Mānuka - Mānuka Vs. Mānuka - *Dracophyllum*	1	0.041	0.245	0.030	0.912
Mānuka - Mānuka Vs. Mānuka - Broom	1	0.451	4.331	0.351	**0.004**
Mānuka - Heather Vs. Mānuka - *Dracophyllum*	1	0.602	4.077	0.338	**0.009**
Mānuka - Heather Vs. Mānuka - Broom	1	0.510	6.137	0.434	**0.007**
Mānuka - *Dracophyllum* Vs. Mānuka - Broom	1	0.361	2.544	0.241	**0.034**

Vs. = versus

Chapter 7

General discussion

7.1 Main findings

Many ecosystems face threats posed by invasive plants. This applies to the Central Plateau, North Island, New Zealand, where several invasive plants have been introduced. While a few studies have reported adverse effects of invasive plants on native species in the area, none have investigated the chemical behaviour of invasive plants and how this may contribute to their competitive success. The present study is novel because it is the first to 1) document volatile emissions from the invasive plant *Calluna vulgaris* (heather) in New Zealand, and the factors regulating emissions under field conditions, 2) measure the VOC emissions of the native shrub, *Leptospermum scoparium* (mānuka), and how these change in a plant invasion scenario and 3) report the chemical responses of an invasive plant to feeding damage (in this case from a specialist herbivore imported into New Zealand as a biocontrol agent for heather).

In Chapter 3, data show that environmental conditions, particularly soil composition, are important drivers of VOC emissions from heather. Emissions were noticeably lower at the site where heather co-occurred with *Cytisus scoparius* (broom), which is another aggressive exotic invader capable of altering soil properties (Caldwell, 2006). This is consistent with literature reports of changes in plant volatile emissions in response to soil nutrient availability (Gouinguené & Turlings, 2002; Veromann et al., 2013).

Under similar field conditions (Chapter 5), it was found that mānuka VOC emissions varied between sites, with a lower amount of VOCs emitted at sites where mānuka co-occurred with invasive species (heather and broom). There also appeared to be environmental variables impacting on VOC emissions from mānuka that were driven by seasonality. Air temperature, herbivory and soil nutrients were the main factors influencing VOC emissions. The impacts of these environmental factors on VOC emission have been documented in previous reports (Holopainen & Gershenzon, 2010; Copolovici & Niinemets, 2016). Interestingly, there were also measured volatile compounds whose emissions were not affected by environmental variables but varied significantly between the sites, suggesting perhaps that other factors including the species composition of nearby plants may be driving emission production as shown in other species (Kigathi et al., 2019).

When experimentally investigating the effects of abiotic (UV-radiation) and biotic (herbivory) factors on VOC production from heather (Chapter 4), lower amounts of VOCs were produced, particularly aldehydes and sesquiterpenes, under higher UV-radiation compared with attenuated UV, and feeding damage by the heather beetle resulted in higher production of terpenoids and lower amounts of green leaf volatiles. This response of heather to its specialist herbivore was also shaped by the plant's phenology, growth stage and abundance of the beetle. These results contradict the findings of some previous studies reporting increased VOC emissions under higher UV levels (Gil et al., 2013; Maja et al., 2016) and augmented levels of green leaf volatiles in response to

herbivory (Schaub et al., 2010; Sufang et al., 2013). These results suggest there may be species-specific responses to stressors or that complex interactions among biotic and abiotic factors, plants' physiology, duration and intensity of stressors and history of exposure could account for the range of responses reported in the literature.

Regarding the ecological effects of invasive VOCs on other community members, a small laboratory bioassay suggests that invasive plant VOCs may have a phytotoxic effect on the germination and growth of mānuka seeds (Appendix 5). Also, the observed changes in arthropod community composition relating to exotic weed invasion suggest that VOC-mediated plant arthropod interactions could be severely impacted during a weed invasion (Chapters 3, 5 and 6).

In summary, this thesis illustrates the complexities in plant volatile emissions during plant invasions and suggests that the presence of invasive plants could create an entirely new chemical environment. The study also hints to the potential ecological effects of invasive plants' volatiles on other plants through allelopathy and disrupting the communication between native plants and associated arthropods.

7.2 Integrating results from all research chapters

In the following section, the links between research chapters will be discussed under four headings; 1) the effect of environmental and seasonal variation on VOC emissions, 2) the impact of neighbouring plants on VOC emissions, 3)

direct ecological impacts of invasive plants' VOCs on other plants – allelopathic properties and 4) indirect ecological impacts of invasive plants' VOCs on other plants – changes in arthropod communities and plant-arthropod interactions.

7.2.1. The effect of environmental and seasonal variation on VOC emissions

Invasive plant species can alter their environment, triggering native plants to respond by changing their VOC emissions. A comprehensive review suggests that invasive plants can increase N availability, modify N fixation rates, increase C stores and increase production of litter which all results in higher decomposition rates (Ehrenfeld, 2003). This is consistent with the increased levels of N, C and other organic matter in soils at heather and broom-invaded sites in this study. This modification in soil properties by invasive species may change the biochemistry of plants through the regulation of substrates required to synthesise compounds. The present study shows a significant effect of soil nutrients on the volatile emissions from both heather and broom (Chapter 3 and 5), as previously demonstrated in other species (Gouinguené & Turlings, 2002; Chen et al., 2008; Ibrahim et al., 2008; Holopainen & Gershenzon, 2010). Results from this study also show an effect of soil water availability on plant volatile emissions, however this was only observed in mānuka in winter. Heather was not tested in winter and would merit further investigation to see if the response is the same as in mānuka. Soil water availability did not affect the volatile emissions of either mānuka or heather in summer when other variables like high temperature and herbivory did.

Although not directly induced by invasion, temperature and UV radiation are also known to influence biogenic volatile organic compound emission (Holopainen & Gershenzon, 2010; Valolahti et al., 2015; Peñuelas & Staudt, 2010; Escobar-Bravo et al., 2017). Several studies have documented increased plant volatile emissions in response to elevated temperature (Holopainen & Gershenzon, 2010; Valolahti et al., 2015), which is consistent with the present study (Chapters 3 and 5). Conversely, the available evidence for plant volatile emissions under elevated UV is relatively limited and inconclusive, with opposing results (Llusia et al., 2012; Maja et al., 2016; Machado et al., 2017). Although UV affected the VOC emissions of heather in the current study (Chapter 4), the results contradict most previous studies that showed increased emissions under high UV levels. Our results may be due to species-specific responses to UV radiation, differences in UV doses, duration of exposure and the conditions of plants prior to experiments.

Changes in environmental factors could influence the production of chemical compounds by invasive plants, which may worsen their impact on natives (Wang et al., 2010). On the other hand, environmentally-induced changes in VOCs from native plants could negatively impact exotic species preventing them from becoming invasive. In this study, mānuka produced higher amounts of β-pinene, 2-heptanone, decanal and (Z)-3-hexenol at the site where it co-occurred with invasive broom. These compounds are known to have phytotoxic effects on other plant species, modify insect behaviour and induce priming in neighbouring plants (Rieth et al., 1986; Naik et al., 2002; Chowhan et al., 2011; Wei & Kang,

2011; Amri et al., 2012; Areco et al., 2014). However, despite increased emissions, broom generally outcompetes mānuka suggesting that these compounds are either not harmful to broom or that their effects are moderate. Therefore, future studies should test whether different concentrations of these compounds have direct or indirect effects on the invasive species. Such information may support the identification and development of potential bioherbicides.

7.2.2. The impact of neighbouring plants on VOC emissions

Emission of volatile organic compounds from mānuka and heather differed significantly between sites depending on the composition of dominant woody species (i.e. conspecifics, native or invasive) and the associated environmental changes. Interestingly, both plants produced lower amounts of VOCs in heterospecific stands, particularly when surrounding vegetation was dominated by an invasive plant species (Chapter 3 and 5).

A similar trend of reduced plant volatile emissions in heterospecific stands has been reported for other species (Peñuelas & Llusià, 1998; Ormeno et al., 2007), suggesting that a reduction in VOC emission may be a widespread response of plants to interspecific competitors. Plants may emit higher concentrations of VOCs in conspecific stands for different reasons, including conspecific cooperation, less competition pressure, reducing the risk of herbivory, and enhanced pollinator attraction. These possibilities will be explored below.

Plants can use VOCs and other sources of information as neighbour detection cues to identify potential competitors and ‘decide’ whether, and how, to respond (Kegge & Pierik, 2010; Ninkovic et al., 2016). A recent study showed that VOCs produced by spotted knapweed (*Centaurea stoebe*) have a neutral to positive effect on neighbouring plants, which demonstrates that volatile compounds can have a facilitative role (Gfeller, 2019). Similarly, volatile chemicals produced by *Artemisia herba-alba* promoted the growth of *Pinus halepensis* seedlings (Arroyo et al., 2018). It is therefore conceivable that upon establishing their neighbour's identity, plants may increase the emission of VOCs in conspecific stands for the common good but reduce emissions to deprive aggressive heterospecific neighbours of such benefits.

The production of plant secondary metabolites comes with a cost, which will determine whether plants prioritise resources to growth, reproduction, or defence (Herms & Mattson, 1992; Gershenzon, 1994). The production of terpenoids, for instance, requires substantial raw materials and biosynthetic enzyme costs, and plants may reduce costs by utilising individual compounds in multiple roles or by catabolising compounds that are not needed (Gershenzon, 1994). Considering such costs associated with their synthesis, when faced with aggressive heterospecific competitors, plants may allocate resources to higher growth rate required to compete, thereby reducing VOC emission.

It has been proposed that herbivore densities will be higher in monospecific plant stands leading to higher emissions as a defensive mechanism (Agrawal et al., 2006). Consistent with this hypothesis, there was a higher number of mānuka

beetles (*Pyronota festiva*) on mānuka in conspecific stands, which corresponded with relatively higher levels of feeding damage (Chapter 5). In response to feeding damage, plants could emit higher amounts of volatiles to attract predators and parasitoids of the herbivores or to directly repel them (Bernasconi et al., 1998; De Moraes et al., 2001; McCormick et al., 2012; Turlings & Erb, 2018), resulting in increased total emissions in conspecific stands. Heather, on the other hand, experiences less pressure from chewing herbivores on the Central Plateau due to patchy distribution of its specialist herbivore in the area. Nevertheless, heather harboured higher numbers of thrips (pollen feeders) in conspecific stands, possibly leading to the measured increase in emissions of fatty acid derivatives and homoterpenes (Chapter 3 and 4). Simultaneously, damage by its specialist herbivore resulted in the release of more terpenoids in dense conspecific stands (Chapter 4).

Floral volatiles are well-known to attract potential pollinators (Krug et al., 2018; Haber et al., 2019). Both mānuka and heather emitted significantly lower amounts of VOCs when neighbour plants were invasive (Chapter 3 and 5). Invasive plants can negatively affect insect pollinator visitation (Morales & Traveset, 2009) or specificity (Lopezaraiza-Mikel et al., 2007) on neighbouring native plants Therefore, native plants in the monospecific stand could benefit from releasing more volatiles to enhance the attraction of pollinators (cooperation) but reduce emissions when paired with heterospecifics to avoid being over-exploited by non-specialised pollinators or nectar robbers.

7.2.3. Direct ecological impacts of invasive plants' VOCs on other plants allelopathic properties

In plant invasion scenarios, the most studied mechanism concerning volatile emissions is allelopathy; whereby the invaders release compounds capable of inhibiting the growth of nearby native plants aiding to their invasion. Examples include but are not limited to the reduction of *Solidago canadensis*'s biomass by the monoterpenes of the invasive *Artemisia vulgaris* (Barney et al., 2009) and reduced establishment of Australian native plants as a result of the allelopathic properties of sesquiterpenes emitted by the invasive exotic plant *Chrysanthemoides monilifera* (Ens et al., 2009).

The results of a small experiment (Appendix 5) showed that aboveground volatiles from heather and broom might be detrimental to mānuka, causing a delay in seed germination and reduction in seedling height and biomass, which is in agreement with reports on other species (Nishida et al., 2005; Araniti et al., 2017). Further experiments with enough replications are required to validate and expand this finding.

It is important to consider that phytotoxicity may not be restricted to invasive plants only. For instance, mānuka released significantly higher emissions of α-pinene and eucalyptol in winter at the site where it co-occurred with *Dracophyllum* (Chapter 5), and these compounds are known for their phytotoxic activity in other systems (Nishida et al., 2005). Therefore, it would be of interest to investigate whether VOCs emitted by native plant have phytotoxic effects on other native plants.

7.2.4. Indirect ecological impacts of invasive plants' VOCs on other plants changes in arthropod communities and plant-arthropod interactions.

Changes in volatile emissions may also impact co-occurring plants indirectly by modifying the behaviour of arthropods. There is evidence of VOCs attracting pollinators (Shuttleworth & Johnson, 2009) and directly repelling herbivores (De Moraes et al., 2001) and attracting natural enemies of herbivores that are using host plant chemicals as cues to locate their prey (Mumm & Hilker, 2005). These interactions can also be disrupted due to the presence of non-host plants (Mauchline et al., 2005; Zhang & Schlyter, 2010; Wang et al., 2016), which may, in turn, impair some ecological services. Plausible explanations for the disruption include non-host plants producing more attractive smells or altering the intensity of the signal required for arthropods to locate their host. Unfortunately, these mechanisms have not yet been studied in relation to plant invasion, so further research on this would be useful.

Arthropods are often impacted during plant invasion partly due to their strong connection with plants (Schirmel et al., 2016; Yekwayo et al., 2016). Results from this study demonstrate significant variation in arthropod community composition between sites invaded by exotic weeds and where native plants are dominant (Chapter 3, 5 and 6). The two exotic invasive plants (heather and broom) have an opposing impact on arthropod community. Broom invasion was often characterised by increased abundance of arthropod groups, while heather was associated with a reduction, reflecting the difficulties in generalising the effect of exotic weeds on arthropod communities. Generally, the presence of the

invaders was linked to a higher number of thrips, detritivores, fungivores and flies (Diptera).

Several studies have also reported changes in arthropod communities in response to plant invasion (Simao et al., 2010; Litt et al., 2014; van Hengstum et al., 2014). These changes are caused by the availability of native food sources, breeding sites, and habitat quality (Herrera & Dudley, 2003; Gratton & Denno, 2006; Wu et al., 2009). Some arthropods, including predators, detritivores and generalist herbivores, may benefit from changes in vegetation structure, increased litter decomposition and food source diversification during plant invasions (Pearson, 2009; Litt et al., 2014; Dudek et al., 2016).

Arthropods show differences in preference associated to the changes in VOC emission by their host plants. For instance, some phytophagous insects are attracted to VOC of plants infested by conspecifics as they may indicate a suitable food source, yet this preference can turn into avoidance when VOCs indicate high competition for resources (Martini et al. 2014; Sun et al. 2014; McCormick et al; 2016). This suggests that insects can use VOCs in host-quality assessment. In an invasion scenario, changes in VOC emissions by the insect's host plant may make them more attractive or repellent to herbivores and their natural enemies, thus impacting arthropod communities.

Variation in arthropod communities observed in the present study may be associated to differences in the chemical behaviour of their host plants but also to overall changes in the chemical environment. Local VOC pools depend on the

plant species composition (Dukes & Mooney, 2004), thus invasive species can strongly modify the chemical environment navigated by insects. In these circumstances, the new chemical environment may disrupt the chemical communication between native plants and their associated arthropods, making it more difficult for them to locate host plants. New chemical environments may also attract other arthropod species intensifying the competition for food and habitats or increasing predation, thereby changing community composition. Future studies should explore the impacts of new chemical environments on insect behaviour to increase our understanding of plant-insect interactions under invasion scenarios and inform conservation efforts.

7.3 Conclusion and future prospects

Despite extensive research on plant invasions and their impacts on economies and biodiversity, the chemical mechanisms behind successful invasions remain poorly understood. This includes emission of volatile compounds from invasive plants and their impact on native plant and animal communities. All plants produce volatile organic compounds that play crucial roles in plant communication. However, the synthesis and release of these compounds are highly dependent on the surrounding environment. Therefore, measuring plant volatile emissions in the field under invasion scenarios could help us to understand the factors regulating VOC emissions and their direct and indirect effects on native species, which could inform weed management strategies.

This study suggests that alongside environmental factors, the composition of plant communities may play a role in regulating VOC emissions. Both native

and invasive plants had higher VOC emissions in conspecific stands than in heterospecific stands. Lower emissions when growing with heterospecific plants may indicate a need to invest more resources in competition, but also to reduce apparency to herbivores, or adjust to environmental changes induced by neighbouring plants. Higher emissions when growing with conspecifics may increase pollinator attraction or VOC-mediated defence strategies, promoting conspecific cooperation. Future studies are therefore recommended to test these hypotheses to enhance our understanding of the dynamics of plant competition in nature. Such studies could include a wider range of soil types in both laboratory and field experiments using various approaches, including transplanting/replanting. Studies should be performed over multiple seasons using different plant species to determine whether these phenomena are widespread among plants. Future studies should also perform more detailed classification of arthropods to explore key species that are more vulnerable or impacted by VOC emissions during plant invasion. For an economically important plant such as mānuka, the information can also be used by people interested in the plant's improvement and cultivation, by identifying favourable traits that could be improved through artificial selection programmes (e.g., plants producing certain compounds that attract pollinators, reduce herbivores or negatively impact invasive plants).

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Appendix 5: Potential allelopathic effects of foliar volatiles of two invasive species

Abstract

Exotic invasive plants, including *Calluna vulgaris* (Heather) and *Cytisus scoparius* (Scotch broom), threaten the survival of native species on New Zealand's Central North Plateau. However, knowledge of the mechanisms contributing to their success in the region is still limited. In this work, we investigated the allelopathic properties of the volatile organic compounds (VOCs) produced by the aerial parts of heather and broom on the seed germination, and seedling growth and biomass production of a native plant mānuka (*Leptospermum scoparium*). Using a twin-chamber cage, mānuka seeds were separately exposed to clean air, aboveground VOCs of conspecifics or volatiles produced by one of the two exotic weeds. The number of germinated seeds were counted daily, and seedlings height measured weekly. Seedlings were harvested after five weeks of exposure, and oven-dried to estimate biomass. The preliminary results demonstrate potential adverse effects of the volatiles produced by heather and broom on seed germination, seedling height and biomass of mānuka. The results suggest that heather and broom may enhance their invasion on the Central North Plateau by releasing volatile compounds that negatively affect the germination and growth of native plants. We, therefore, recommend further studies with additional replicates to support this observation and expand the present findings.

Introduction

Introduction of plants into a non-native range is a major challenge facing different ecosystems as most introduced plants are able to establish, suppress natives and modify their new region (Stinson et al., 2006). The threat posed by invasive alien species in a new region is only second to direct habitat destruction, contributing to high biodiversity and economic losses (Pimentel et al., 2005). Most exotic invasive weeds have high phenotypic plasticity that promotes their success in new environments (Funk, 2008; Van Kleunen et al., 2010).

The novel weapon hypothesis also predicts that invasive plants may enhance their competitiveness in new habitats by releasing allelochemicals, i.e., compounds that influence the germination, growth, survial or reproduction of other species, into the environment (Callaway & Ridenour, 2004). This phenomenon has been extensively studied regarding aqueous extracts from roots and leaves (Bais et al., 2003; Thorpe et al., 2009; Wu et al., 2009; Hu et al., 2013), yet less is known about the allelopathic properties of airborne chemicals, i.e., volatile organic compounds (VOCs). Some studies have demonstrated that volatile compounds released into new environments by exotic invasive plants can have allelopathic properties, influencing native species negatively (Wang et al., 2010; Inderjit et al., 2011; Tang et al., 2019). This evidence suggests that VOC-mediated allelopathy could be part of the competition strategy of invasive plants.

Calluna vulgaris (Heather) and *Cytisus scoparius* (Scotch broom; henceforth broom) are both originally from Europe but are highly invasive on the Central

North Plateau of New Zealand. These exotic weeds pose a major threat to the environment they invade by modifying soil properties, altering arthropod composition and outcompeting native plants (Keesing, 1995; Blayney, 2012; Effah et al., 2020). Both plants are rich VOC emitters (Pardo-Muras et al., 2018; Effah et al., 2020), but only Pardo-Muras and co-workers (2018) has explored the allelopathic properties of broom volatiles and there are no reports for heather.

Therefore, the present study aimed to elucidate potential allelopathic properties of the aboveground volatiles produced by heather and broom. This was achieved through a germination experiment in which the seeds of a common New Zealand native competitor *Leptospermum scoparium* (mānuka) were separately exposed to the foliar volatiles of mānuka, heather and broom in a twin-chamber cage. Germinated seeds, seedling heights and biomass, were measured. Based on the literature, we expect the effects of volatiles on seedlings to differ between the three emitting plants, with VOCs of the heather and broom being more detrimental compared to that of mānuka.

Materials and methods

This study was conducted in a temperature (25° C) and humidity (46%) controlled room, with 16 hours of light and 8 hours dark period.

Full (viable) mānuka seeds (Fig. 1) were planted in a Petri dish containing volcanic soil collected from the Central North Plateau and exposed to one of the four treatments; 1) clean air, 2) volatiles of mānuka, 3) volatiles of heather and 4) volatiles of broom in a twin chamber growth cage (Fig. 2). Thirty Petri dishes (10 seeds per dish) were placed in one chamber of the growth cage, and two of

the tested plants (undamaged) placed in the other chamber. Air was pulled from the chamber containing the undamaged plants (2.2 ms^{-1}) into the chamber with Petri dishes. As a control, air was pulled from one chamber without plants into the other chamber containing Petri dishes. Lids of the Petri dishes had tiny holes to allow air passage (Fig. 2).

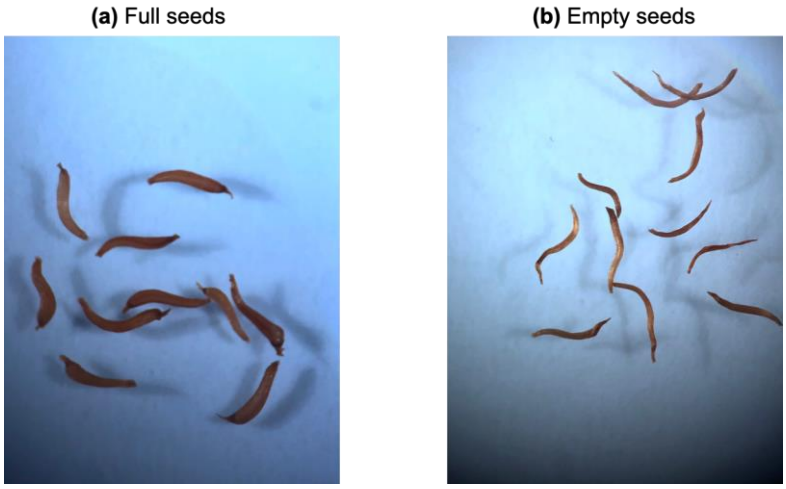

Figure 1. Sorting manuka seeds for experiment. Only full seeds were selected for the experiment.

Seedlings were watered by hand three times per week, and no additional nutrient applied. The number of seeds that germinated was recorded daily, and seedling height measured weekly. After five weeks of exposure, seedlings were harvested and oven-dried at 60º C for 48 hours to estimate biomass.

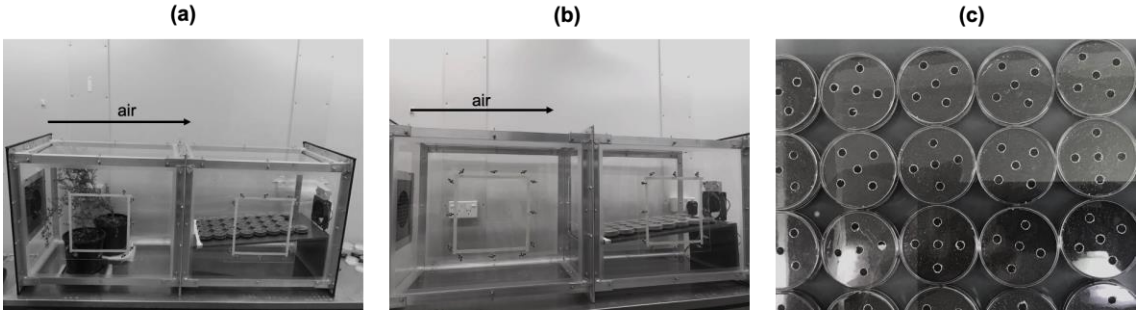

Figure 2. Experimental setup. a) cage with VOC emitting plants in one chamber and Petri dishes in the other chamber, b) cage without emitting plant (control) and c) illustrates holes created on Petri dishes for air passage. Arrow in (a) and (b) shows the direction of airflow in respective cages.

Results

Seeds started to germinate on the fifth day after planting. Germination was delayed for mānuka seeds exposed to the volatiles of broom compared to those exposed to clean air or VOCs from heather or mānuka (Fig. 3a). Seedling height was increased in mānuka seedlings exposed to air and VOCs emitted by mānuka relative to seedlings exposed to the foliar volatiles of the two exotic invasive weeds (Fig. 3b).

Similarly, belowground, aboveground and total biomass were often reduced in mānuka seedlings exposed to VOCs of the exotic weeds. However, root biomass was increased in seedlings exposed to broom's volatiles relative to heather exposed seedlings (Fig. 3).

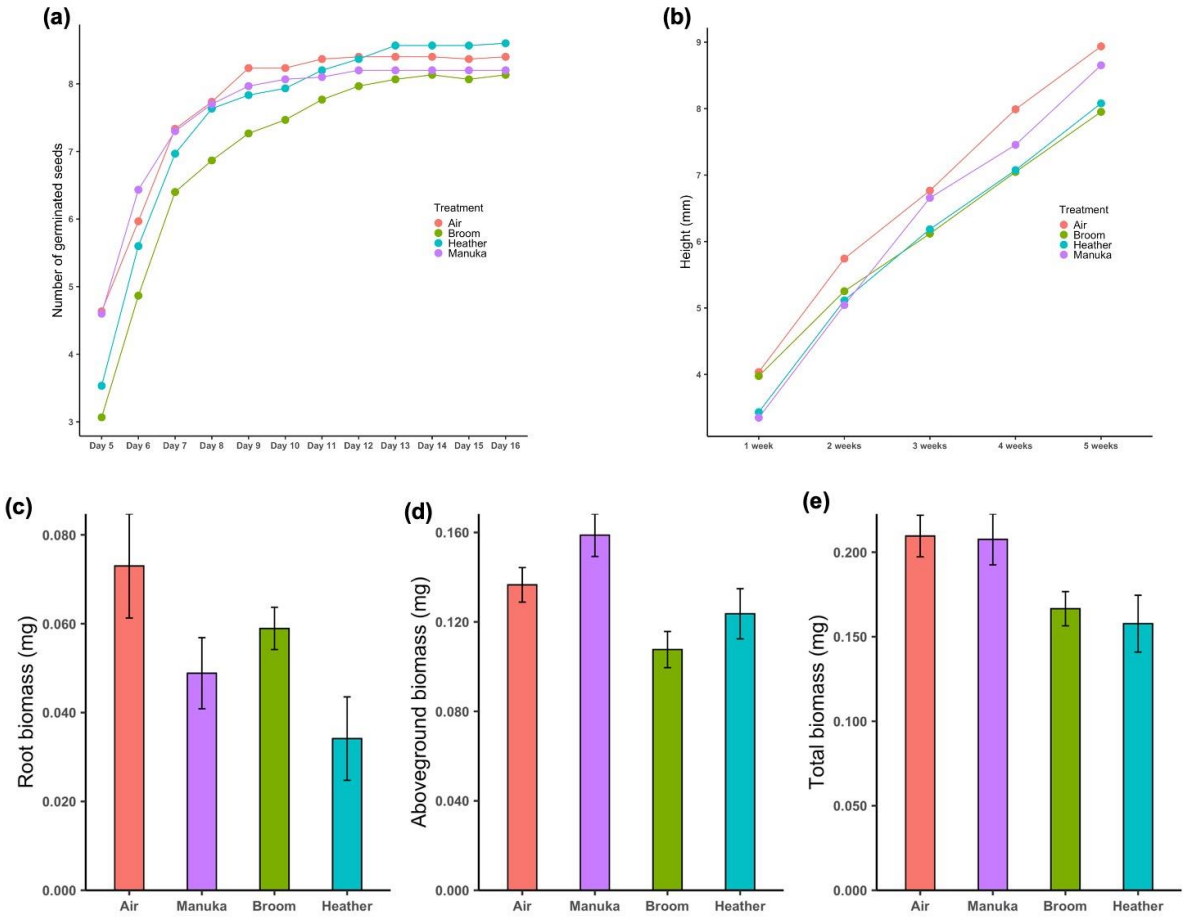

Figure 3. Measurements of seed germination, height and biomass of manuka seedlings exposed to the aboveground volatiles emitted by mānuka, broom or heather. Clean air was used as a control. Y-axis shows mean ± SE of measured variables.

Discussion

This study suggests there is an adverse effect of the aboveground volatiles produced by the exotic invasive plants heather and broom on the seed germination, seedling height and biomass of New Zealand native mānuka plants, and suggest that besides direct competition for resources, aboveground volatile emissions might contribute to the dominance of these weeds in the environment they invade.

Consistent with the present study, other studies have demonstrated that invasive plants often utilise this mechanism to enhance their dominance in their invaded regions. For instance, monoterpenes emitted by the invasive *Artemisia vulgaris* reduced the aboveground biomass of native *Solidago canadensis* by 50% (Barney et al., 2009), while sesquiterpenes emitted by the exotic bitou bush (*Chrysanthemoides monilifera*) had a negative effect on the seedling growth of native *sedge* (*Isolepis nodosa*) (Ens et al., 2009). A more recent study investigating the allelopathic potential of the invasive plant *Xanthium sibiricum* also found that VOCs released by *X. sibiricum* exhibits inhibitory activities, suppressing the root growth of receiver plants (Tang et al., 2019), suggesting that allelopathy may be widespread during plant invasion.

Both individual compounds (Wang et al., 2010) and volatile blends (Barney et al., 2009; Araniti et al., 2017) can be phytotoxic to receiver plants, with their effects likely to be more harmful in response to climate change (Wang et al., 2010). In the present study, mānuka seeds were exposed to the full volatile blend of emitter plants, and the observed inhibitory activities may, therefore, be a combined effect of the blend. Both heather and broom are rich producers of terpenoids (Pardo-Muras et al., 2018; Effah et al., 2020), with most of these compounds shown to have allelopathic properties in other systems (Nishida et al., 2005; Pardo-Muras et al., 2018; Pardo-Muras et al., 2019). It is also possible that only certain individual compounds in the bouquet of volatiles contributed to the inhibitory activities in the present study, with other compounds having neutral to positive effects, creating an avenue for further research.

In summary, this work explored the potential allelopathic properties of the aboveground volatiles produced by exotic invasive plants heather and broom on mānuka. The preliminary results show that VOCs produced by the two exotic weeds may inhibit the growth of receiver mānuka plants, and support the claim that invasive plants release alellopathic compounds into the host range, which may contribute to their success in naïve environments (Callaway & Ridenour, 2004). While allelopathic properties of VOCs produced by broom has been reported (Pardo-Muras et al., 2018; Pardo-Muras et al., 2019), this study is the first to test such properties in heather.

Because of the impacts of COVID-19 lockdowns, we were unable to replicate the treatments, which limits the findings from this study. We, therefore, recommend further studies to test these interactions to validate and expand on these findings. Future studies could also include volatile measurement of heather and broom plants, tests with individual compounds, and explore whether the native plants' VOCs also have allelopathic properties against the invaders.

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Appendix 6: Statement of Contribution

Appendix 6.1 Contribution for Chapter 2

DRC 16

MASSEY UNIVERSITY
UNIVERSITY OF NEW ZEALAND
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Evans Effah
Name/title of Primary Supervisor:	Dr Andrea Clavijo McCormick
In which chapter is the manuscript /published work:	2

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Effah, E., Holopainen, J.K. and McCormick, A.C., 2019. Potential roles of volatile organic compounds in plant competition. Perspectives in Plant Ecology, Evolution and Systematics 38, 58-63.

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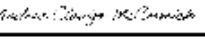
Primary Supervisor's Signature:	
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Appendix 6.2 Contribution for Chapter 3

DRC 16

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Evans Effah
Name/title of Primary Supervisor:	Dr Andrea Clavijo McCormick
In which chapter is the manuscript /published work:	3

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Effah, E., Barrett, D.P., Peterson, P.G., Godfrey, A.J.R., Potter, M.A., Holopainen, J.K. and Clavijo McCormick, A., 2020. Natural Variation in Volatile Emissions of the Invasive Weed Calluna vulgaris in New Zealand. Plants 9, 283.

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Date:	17-Jun-2020
Primary Supervisor's Signature:	Andrea Clavijo McCormick
Date:	18.06.2020

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Appendix 6.3 Contribution for Chapter 4

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In which chapter is the manuscript /published work:	4

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(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Effah, E., Barrett, D.P., Peterson, P.G., Wargent, J.J., Potter, M.A., Holopainen, J.K. and Clavijo McCormick, A., 2020. Herbivory and Attenuated UV Radiation Affect Volatile Emissions of the Invasive Weed Calluna vulgaris. Molecules 25, 3200.

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Date:	13-Oct-2020
Primary Supervisor's Signature:	Clavijo-McCormick, Andrea Digitally signed by Clavijo-McCormick, Andrea Date: 2020.10.13 15:20:22 +13'00'
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Appendix 6.4 Contribution for Chapter 5

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In which chapter is the manuscript /published work:	5

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- Please provide the full reference of the Research Output:

Effah, E., Barrett, D.P., Peterson, P.G., Potter, M.A., Holopainen, J.K. and McCormick, A.C., 2020. Seasonal and environmental variation in volatile emissions of the New Zealand native plant Leptospermum scoparium in weed-invaded and non-invaded sites. Scientific reports, 10, 1-11.

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Date:	13-Oct-2020

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Appendix 6.5 Contribution for Chapter 6

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In which chapter is the manuscript /published work:	6

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Effah, E., Barrett, D.P., Peterson, P.G., Potter, M.A., Holopainen, J.K. and Clavijo McCormick, A., 2020. Effects of Two Invasive Weeds on Arthropod Community Structure on the Central Plateau of New Zealand. Plants 9, 919.

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Date:	13-Oct-2020
Primary Supervisor's Signature:	Clavijo-McCormick, Andrea Digitally signed by Clavijo-McCormick, Andrea Date: 2020.10.13 15:28:22 +13'00'
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