Soil warming increases plant species richness but decreases germination from the alpine soil seed bank

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Abstract

Global warming is occurring more rapidly above the treeline than at lower elevations and alpine areas are predicted to experience above average warming in the future. Temperature is a primary factor in stimulating seed germination and regulating changes in seed dormancy status. Thus, plant regeneration from seed will be crucial to the persistence, migration and post disturbance recruitment of alpine plants in future climates. Here, we present the first assessment of the impact of soil warming on germination from the persistent alpine soil seed bank. Contrary to expectations, soil warming lead to reduced overall germination from the soil seed bank. However, germination response to soil temperature was species specific such that total species richness actually increased by nine with soil warming. We further explored the system by assessing the prevalence of seed dormancy and germination response to soil disturbance, the frequency of which is predicted to increase under climate change. Seeds of a significant proportion of species demonstrated physiological dormancy mechanisms and germination of several species appeared to be intrinsically linked to soil disturbance. In addition, we found no evidence of subalpine species and little evidence of exotic weed species in the soil, suggesting that the soil seed bank will not facilitate their invasion of the alpine zone. In conclusion, changes in recruitment via the alpine soil seed bank can be expected under climate change, as a result of altered dormancy alleviation and germination cues. Furthermore, the alpine soil seed bank, and the species richness therein, has the potential to help maintain local species diversity, support species range shift and moderate species dominance. Implications for alpine management and areas for further study are also discussed.

Keywords: alpine, Australia, biodiversity, climate change, dormancy, global warming, soil seed bank

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Introduction

Seeds of many species possess the means to postpone germination and persist in a soil seed bank. Alpine soil seed banks have historically been considered insignificant or non-existent and alpine plant reproduction was assumed to be primarily vegetative (Billings & Mooney, 1968; Bliss, 1971). However, the importance of sexual reproduction in the maintenance of alpine vegetation is continually reinforced by *in situ* observations of seed germination (Schlag & Erschbamer, 2000; Forbis, 2003; Körner, 2003; Erschbamer *et al.*, 2008). In addition, sizable seed banks have been found to exist in alpine soils all over the world (Archibold, 1984; McGraw & Vavrek, 1989; Diemer & Prock, 1993; Arroyo *et al.*, 1999; Cavieres & Arroyo, 2001; Welling *et al.*, 2004; Klug-Pumpel & Scharfetter-Lehrl, 2008; Marcante *et al.*, 2009; Venn & Morgan, 2010; Li *et al.*, 2011). Indeed,

more than half of alpine species present in the standing vegetation may exist in the soil as seeds (McGraw & Vavrek, 1989). Cold, exposed and unstable environmental conditions characteristic of alpine habitats favour 'persistent' soil seed banks in which seeds remain viable for two or more years, as opposed to 'transient' seed banks that only endure until the following growth season (Thompson & Grime, 1979; Walck *et al.*, 2005). Previous work has uncovered persistent soil seed banks in the Australian Alps, with seed densities comparable to those previously reported for subarctic Finland, the High Arctic Svalbard, the high alpine region of the Chilean Andes and tundra habitats of northern Alaska (Venn & Morgan, 2010).

The roles of soil seed banks in alpine habitats are numerous and varied. Alpine plant recruitment occurs in an environment characterized by low temperatures and short growing seasons (Billings & Mooney, 1968; Bliss, 1971). By persisting in the soil, dispersed seeds

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can bet-hedge against temperature and rainfall events that could trigger germination, but would not support seedling growth and survival to reproductive maturation (Venable & Brown, 1988; Philippi, 1993; Simons & Johnston, 2006). Soil seed banks function as a genetic memory of a population, enabling species to retain some propagules in reserve (Brown & Venable, 1986; Levin, 1990; Arroyo *et al.*, 1999), and counteract the annual fluctuations in alpine seed production and quality (Chambers, 1995; Dullinger & Hulber, 2011). Seed banks also play an important role in community dynamics, particularly the re-establishment of populations following disturbance events (Bakker *et al.*, 1996; Funes *et al.*, 2001; Fenner & Thompson, 2005). Freeze/thaw action, fire and soil erosion, are expected to increase in frequency in alpine habitats under future climates (Worboys & Good, 2011), and will put soil seed banks under increasing pressure during the re-recruitment episodes that follow. Finally, seeds in the soil seed bank represent not only the standing vegetation, but dispersants from other areas, and therefore have the potential to provide the colonizers necessary for species distribution shifts.

The ability of seeds to persist in soil depends upon many factors, including seed size and morphology (Thompson *et al.*, 1993; Bekker *et al.*, 1998; Cerabolini *et al.*, 2003), seed longevity (Long *et al.*, 2008) and seed dormancy (Thompson *et al.*, 1993; Cavieres & Arroyo, 2001; Fenner & Thompson, 2005). Physiological dormancy (PD) enables freshly dispersed seeds to postpone germination despite adequate temperature and moisture conditions (Vleeshouwers *et al.*, 1995; Benech-Arnold *et al.*, 2000). The majority of seeds with PD have what is termed a 'non-deep' or 'intermediate' level of PD that, unlike 'deep' PD, can be artificially by-passed by the application of the plant growth hormone gibberellic acid (GA$_3$, Baskin & Baskin, 2004). Indeed, freshly dispersed seeds of numerous alpine species reportedly possess PD that can be bypassed with GA$_3$ and/or alleviated by cold stratification pre treatments (sometimes referred to as moist after-ripening; Mattana *et al.*, 2009; Mondoni *et al.*, 2009; Venn & Morgan, 2009; Schwienbacher *et al.*, 2011). Thus, comparison of soil treated with GA$_3$ to soil without added hormone may reveal not only a larger portion of the germinable soil seed bank, but also the extent to which seeds have PD (e.g. Bueno *et al.*, 2011). Despite this, the majority of germinable soil seed bank studies do not consider the role of PD and are therefore likely to have underestimated both seed density and the role of PD in seed bank formation and persistence.

Evidence from Scandinavia, Europe, Central Asia and the Americas suggests that warming associated with climate change is occurring more rapidly above the treeline than at lower elevations, therefore alpine areas are predicted to experience above average warming in the future (Diaz & Bradley, 1997; Beniston, 2003; Kullman, 2004). In Australia, alpine areas have seen a rise in air temperature of ca. 0.2 °C per decade over the last 35 years (Hennessy *et al.*, 2003; Green & Pickering, 2009), and mean temperature predictions for 2050 lie between +0.6 and +2.9 °C relative to 1990 (Hennessy *et al.*, 2003). Although soil temperature data are limited for the Australian Alps, elsewhere in Australia maximum soil temperatures are predicted to increase by almost double that of air temperature by the latter half of the 21st Century (Ooi *et al.*, 2009). Temperature is a primary factor in stimulating germination and regulating changes in dormancy status (Benech-Arnold *et al.*, 2000; Vleeshouwers & Bouwmeester, 2001). Soil warming has been predicted to contribute to net losses to soil seed banks in Canadian boreal wetland and arid zone Australia, through increased germination and/or reduced viability of the seeds therein (Hogenbirk & Wein, 1992; Ooi *et al.*, 2009). Thus, it seems likely that altered temperature regimes will affect germination from alpine soil and impact upon the response of alpine plants to climate change, including distribution shifts and recruitment post disturbance. In addition, fresh alpine/tundra seeds have increased and/or accelerated in germination when sown at increased incubation temperatures (Sayers & Ward, 1966; Graae *et al.*, 2008; Mattana *et al.*, 2009; Milbau *et al.*, 2009; Mondoni *et al.*, 2012). Despite this, to the best of our knowledge, there are no published studies on the effect of increased temperatures on germination from an alpine soil seed bank.

Here we present the first assessment of the impact of soil warming, as predicted under climate change, on germination from an alpine soil seed bank. Given the documented effects of warming on germination from soil seed banks of other habitats, and on germination of alpine/tundra seeds *ex situ*, we expect germination from the alpine soil seed bank to increase and/or accelerate with soil warming. Furthermore, given the reported success of cold stratification and application of GA$_3$ in achieving germination of alpine seeds, we also expect PD seeds to be present in the soil seed bank and to germinate in response to the application of GA$_3$. Lastly, we expect disturbance of the soil to promote germination from the seed bank therein (Chambers, 1995; Bueno *et al.*, 2011). By investigating these questions we will improve our understanding of the influence of climate change on germination from the alpine soil seed bank and results will support the long-term management of alpine plant biodiversity into the future.

Materials and methods

Study sites and sampling procedures

The Australian Alps occupy ca. 25 000 km^2 of southeast Australia. Soil and vegetation sampling was conducted between 1935 and 1961 m above sea level (a.s.l., 36°27′697″S, 148°19′851″E), in Kosciuszko National Park (KNP). There is an almost complete layer of alpine humus and associated organic soils at the study site, and vegetation is classified as tall alpine herbfield, characterized by species of *Poa* (Poaceae) and *Celmisia* (Asteraceae), and containing many other conspicuous forbs (Costin *et al.*, 2000). Mean 09:00 hours and 15:00 hours air temperatures in KNP during the summer growing season (December to Feb inclusive), are 12.4 °C and 16.4 °C respectively, based on monthly averages recorded between 1966 and 2009 at Thredbo Village (36°50′S, 148°30′E and 1380 m a.s.l.) and Thredbo AWS (36°49′S, 148°29′E and 1957 m a.s.l.) weather stations (Bureau of Meteorology, 2010). Annual precipitation in KNP ranges from 1800 to 3100 mm (60% of which falls as winter snow and persists for at least 4 months, Costin *et al.*, 2000).

Soil was collected in mid-summer 2010, before the onset of seeding of standing vegetation in late summer/autumn, to ensure that seeds in the soil had persisted since dispersal in summer 2009 or earlier. Soil was collected along three parallel transects (150 m in length and ca. 500 m apart), from four locations spaced 50 m apart. The total elevational variation among sample sites was 26 m. A quadrat (1 × 2 m^2) was randomly placed at each sample site and standing vegetation within the quadrat was identified and cover abundance per species visually estimated to the nearest 5%. Approximately 1000 cm^3 of soil was dug out from 16 evenly spaced locations within each quadrat, to a depth of ca. 10 cm, to fill 16 1 L plastic bags. This method ensured broad and even sampling of the entire surface area of each quadrat, as far as the very dense above- and below-ground vegetation would allow. The soil samples were kept between 15 °C and 20 °C during transportation to the laboratory.

Seed germination testing

In the laboratory, all soil samples were air-dried at 15 °C and 15% relative humidity for 6 weeks before being bulked per quadrat and mixed by hand. Large stones and obvious plant matter were removed by hand. Soil from each sample was then divided into four equal parts by mass and spread evenly over 2 cm of sterilized, autoclaved sand in four 300 × 250 mm^2 trays. Each tray therefore represented 0.5 m^2 of alpine surface area. Weed mat was placed in the base of each tray to prevent the loss of soil and sand.

Two trays per sample site were randomly allocated to each of a cool and a warm temperature-controlled glasshouse. Soil temperatures within the cool and warm glasshouses were designed to mimic present-day optimal alpine growing season conditions and a warmer scenario reflective of predicted climate change beyond 2050 respectively. Soil temperatures in the cool glasshouse (mean ± standard error), were 19.2 ± 0.3 °C during the day and 11.9 ± 0.2 °C at night; measured using

Smart Button temperature data loggers (ACR Systems), placed 10 mm below the soil surface. These soil temperatures were created by air temperatures of 20.6 ± 0.02 °C during the day (06.00–17.45 hours), and 11.5 ± 0.01 °C at night (18.00–05.45 hours), which were guided by air temperature data collected in KNP between 1976 and 2009 (as described above, Bureau of Meteorology, 2010).

Soil temperatures in the warm glasshouse were ca. 4 °C warmer than those in the cool glasshouse, both day and night; 23.3 ± 0.3 °C during the day and 15.9 ± 0.1 °C at night. This soil warming was created by air temperatures of 29.8 ± 0.02 °C during the day and 19.2 ± 0.02 °C at night, and fell within the +1.2 to +5.8 °C predicted soil temperature increase for 2050 and beyond (Hennessy *et al.*, 2003; Ooi *et al.*, 2009).

We made the assumption that, due to summer rain, snow melt and the absence of a marked dry season, seeds in the soil *in situ* may be sufficiently hydrated for germination at any stage during the growth season. Therefore, from day six onwards, all trays were watered twice daily with a fine spray-mist of water. Day length was natural (at least 11 h of daylight), until the beginning of winter 2010, after which day lengths were maintained by additional lighting from four halogen lamps (150 w), mornings (06:00–09:00 hours) and evenings (16:00–19:00 hours). All trays were examined weekly (with the exception of week 4), and seedlings were recorded and tagged as soon as they were visible to the naked eye.

Dormancy assessment

Seed germination of a wide range of Australian plant species is promoted by the plant growth hormone gibberellic acid (GA$_3$, Bell, 1999), with application of between 5 mg L^{-1} and 1 g L^{-1} GA$_3$ reportedly enough to initiate a germination response in seeds with physiological dormancy (PD, Turner & Merritt, 2009). To maximize germination and assess the prevalence of seed PD, water containing 200 mg L^{-1} GA$_3$ was administered to one tray of soil from each sample site, within each temperature environment, for the first 5 days, and water without GA$_3$ was added to remaining trays.

Soil disturbance

After 12 weeks, germination rate slowed significantly and at 20 weeks no new seedlings had been recorded for at least 2 weeks. Soil in each tray, including any moss and bryophytes, was turned over with a fork assess whether disturbance (e.g. by animals, erosion, freeze/thaw action, etc.), would result in any further germination. Subsequent germination was scored for a further 10 weeks, by which time there had been no germination in any tray for 2 weeks and the experiment was terminated, 30 weeks after commencement.

Plant identification

Seedlings from the soil seed bank were identified to genus and species as early as possible. Where seedlings could not be identified within the first 3–4 weeks, they were assigned a

morphotype based on obvious family diagnostics. A subset of between 10 and 20 seedlings per morphotype were then potted up into 50 × 50 mm tubes containing seed-raising mix (Debco), and administered with a low phosphorous fertilizer (Osmocote Plus Low P). These seedlings were grown on in the 20/10 °C glasshouse until development of flowers or other diagnostics enabled species identification. As seedlings were identified or allocated a morphotype, they were removed from trays to facilitate further germination. Seedlings that died before they could be identified were recorded as unidentified.

Experimental design and data analysis

The seed germination testing was laid out as a row-column factorial design. In each of the two glasshouses there was an 8 × 3 arrangement of trays for the three treatment factors, i.e. GA_3 (with, without), transect and position along transect. The software package CycDesigN (Whitaker *et al.*, 2009) was used to generate a row-column design that optimized the separation of signal and noise, and randomized trays separately within each glasshouse. As it was not possible to replicate glasshouses, any differences between glasshouses are assumed to be due to temperature.

The density of germinable seeds in the soil (mean ± standard error m^{-2}), was calculated using data from the treatment that yielded the most seedlings, per sample location. Cumulative data for the total number of seedlings emerging from the soil, the number of individuals of the three most common families (Poaceae, Cyperaceae and Juncaceae), and the three most abundant species [*Rytidosperma nudiflorum* (Poaceae), *Carex breviculmis* and *Cyperus flavidus* (Cyperaceae)], were analysed using the statistical package Genstat (14th edition) at 3, 8, 12, 20 and 30 weeks. The count data were analysed as a generalized linear model with Poisson error distribution and log link. Cumulative counts at week 20 (final germination pre disturbance), and 30 (final germination post disturbance), for total seedlings and the three most common families were compared to assess the effect of soil disturbance using a split-plot analysis. The effect of position of germination tray in the experiment (i.e. row and column), was explored in the preliminary analysis and did not explain differences between the two glasshouses. The final model included terms for temperature, GA_3, transect and the interaction between temperature and GA_3, all as fixed effects.

The similarity between the species found to be present in the soil (across all treatments), and the standing vegetation at each sample location, was analysed using Jaccard's qualitative similarity index (SJ, Jongman *et al.*, 1987; Welling & Laine, 2000), and the quantitative similarity ratio (SR, see Ball 1966 in Jongman *et al.*, 1987). The qualitative similarities were calculated for each quadrat according to the equation:

$$SJ = c/(A + B - C) \quad (1)$$

where A is the total number of species in the standing vegetation, B is the total number of species in the germinable soil seed bank, and c is the number of species shared by both the standing vegetation and the soil seed bank

(Welling & Laine, 2000). Non matches in species between the soil seed bank and the standing vegetation ($c = 0$), at the quadrat level, can ultimately bring the site mean down. As such, we also calculated a quantitative index (SR) which was used to give different weights to the presence or absence of a species, with regard to differences in abundance when the species is present (Jongman *et al.*, 1987). These quantitative similarities (the similarity ratio, see Ball 1966 in Jongman *et al.*, 1987), were calculated for each quadrat according to the equation:

$$SR_{ij} = \sum kY_{ki}Y_{kj} / (\sum kY_{ki}^2 + \sum kY_{kj}^2 - \sum kY_{ki}Y_{kj}) \quad (2)$$

where Y_{ki} is the abundance of the kth species in the standing vegetation, Y_{kj} is the abundance of the kth species in the soil seed bank, and $Y_{ki}Y_{kj}$ is the abundance of species shared by the soil seed bank and the standing vegetation. As per Welling & Laine (2000), the values from the soil seed bank and the standing vegetation were transformed to comparable percentages, making the number of germinants and per cent cover of standing vegetation sum to 100 at every sample location. Both indices vary from 0 to 1. Values closer to 1 indicate a close similarity between standing and seedling vegetation, and values closer to 0.1 indicate low similarity.

Results

The total germinable soil seed bank

Due to our regular examination of the relatively shallow soil, it was deemed unlikely that germination occurred and seedlings did not survive to emergence. Therefore, we equate seedling emergence with seed germination. As expected, the germinable alpine soil seed bank was comparable in size and species diversity to that found previously in the Australian Alps (Venn & Morgan, 2010). The density of germinable seeds in the soil (mean ± standard error), calculated using data from the glasshouse treatment that yielded the most seedlings per sample site, was $382 ± 35$ m^{-2}. Of a total 7207 seedlings, 67.5% were identified to species level, 0.24% to genus, but not species and 29.1% just to family (94.6% of the latter seedlings being Poaceae). A further 3.2% of seedlings died before they could be identified. In total, 45 species, representing 22 families, were identified (Table 1). Of the 45 identified species, 30 (representing 95.0% of the total number of seedlings), were considered to be obligate Australian alpine species (Costin *et al.*, 2000). Of the remaining species, four (0.4% of total germinants), were considered to be more prevalent sub alpine (Costin *et al.*, 2000), and five (2.1% of total germinants), were classified as not alpine specific due to their wide distribution within Eastern New South Wales (Plant-NET, 2012). Only five species (2.5% of total germinants), were exotic weeds (Table 1).

Table 1 Total germinable soil seed bank, per soil temperature/gibberellic acid (GA$_3$) treatment, listed as a percentage proportion of the total, from largest to smallest proportion of the total across all treatments. Species identification where known. Values in parentheses are the proportion of germinants that emerged post disturbance. Entries in bold only germinated from soil that was treated with GA$_3$

Family	Genus	Species	Cool without GA$_3$	Cool with GA$_3$	Warm without GA$_3$	Warm with GA$_3$	Total %
Poaceae	*Rytidosperma*	*nudiflorum*[§]	11.82 (9.74)	12.77 (2.61)	3.12 (35.56)	6.37 (8.06)	34.08 (9.12)
Poaceae	Unidentified	Unidentified	7.46 (0.19)	3.82	7.42 (0.75)	8.84 (2.04)	27.54 (0.91)
Cyperaceae	*Carex*	*breviculmis*[§]	3.30 (5.46)	1.82 (11.45)	7.33 (4.92)	3.87 (6.81)	16.32 (6.21)
Juncaceae	*Luzula*	*novae-cambriae*[§]	1.80 (22.31)	0.97 (31.43)	2.12 (26.14)	0.75 (62.96)	5.65 (30.71)
Unidentified	Unidentified	Unidentified	1.23	1.30	0.49	0.15	3.18
Cyperaceae	*Cyperus*	*flavidus*[†]	0.71 (100)	0.44 (100)	0.42 (93.33)	0.35 (100)	1.91 (98.55)
Poaceae	*Trisetum*	*spicatum* subsp. *australiense*[§]	0.43 (6.45)	0.43 (9.68)	0.56 (37.5)	0.46 (9.09)	1.87 (17.04)
Boraginaceae	***Myosotis***	***laxa***[‡]	–	**1.43**	–	**0.35**	**1.78**
Campanulaceae	*Wahlenbergia*	*ceraceae*	0.25 (33.33)	0.11 (62.50)	0.89 (46.88)	0.04	1.29 (44.09)
Ericaceae	Unidentified	Unidentified	–	0.14	0.03	0.51	0.68
Polygonaceae	*Acetosella*	*vulgaris*[‡,§]	–	0.01	0.64 (100)	–	0.65 (97.87)
Cyperaceae	**Unidentified**	**Unidentified**	–	–	–	**0.64 (6.52)**	**0.64 (6.52)**
Portulacaceae	*Neopaxia*	*australasica*	0.24	0.11	0.07 (20)	0.12	0.54 (2.56)
Asteraceae	*Ewartia*	*nubigena*	0.14 (30)	0.11 (25)	0.14 (60)	0.12 (22.22)	0.51 (35.14)
Rubiaceae	*Asperula*	*gunnii*[§]	0.12	0.14	0.10 (85.71)	0.06 (25)	0.42 (23.33)
Brassicaceae	*Cardamine*	*gunnii*[*,§]	0.06	–	0.12 (11.11)	0.14	0.32 (4.35)
Caryophyllaceae	*Scleranthus*	*biflorus*[§]	0.04	0.12	0.07	0.07 (20)	0.31 (4.55)
Thymelaeaceae	*Pimelea*	*alpine*[§]	0.04 (66.67)	0.07 (80)	0.01	0.11 (12.5)	0.24 (41.18)
Fabaceae	*Oxylobium*	*ellipticum*[§]	0.01	–	0.11	0.10	0.22
Violaceae	*Viola*	*bertonicifolia* subsp. *betonicifolia*[§]	0.03 (50)	0.03 (50)	0.07	0.03 (50)	0.15 (27.27)
Geraniaceae	*Geranium*	*antrorsum*	0.03 (50)	0.03	0.06	0.03	0.14 (10)
Myrtaceae	*Baeckea*	*gunniana*[§]	–	0.10	0.01	0.01	0.12
Juncaceae	**Unidentified**	**Unidentified**	–	–	–	**0.11**	**0.11**
Asteraceae	Unidentified	Unidentified	0.04	0.03	–	0.03	0.10
Asteraceae	*Senecio*	*gunnii*	0.01(100)	0.01	0.04 (33.33)	0.01	0.08 (33.33)
Onagraceae	*Epilobium*	Unidentified	0.08 (16.67)	–	–	–	0.08 (16.67)
Asteraceae	***Erigeron***	***nitidus***[§]	–	**0.03**	–	**0.04**	**0.07**
Asteraceae	*Euchiton*	*fordianus*	0.01 (100)	0.03 (100)	0.01 (100)	0.01 (100)	0.07 (100)
Asteraceae	*Euchiton*	*involucratus*[†]	–	–	0.07	–	0.07
Caryophyllaceae	*Scleranthus*	Unidentified	0.07	–	–	–	0.07
Asteraceae	***Senecio***	***pinnatifolius*** var. ***pleiocephalus***[§]	–	–	–	**0.06**	**0.06**
Asteraceae	***Euchiton***	***argentifolius***	–	**0.06**	–	–	**0.06**
Asteraceae	***Euchiton***	***gymnosephalus***[†]	–	**0.04**	–	**0.01**	**0.06**
Caryophyllaceae	*Stellaria*	Unidentified	–	–	0.06	–	0.06
Caryophyllaceae	***Stellaria***	***pungens***[†]	–	–	–	**0.06**	**0.06**
Caryophyllaceae	Unidentified	Unidentified	–	–	–	0.04 (33.33)	0.04 (33.33)
Fabaceae	*Acacia*	*mearnsii*[†]	0.01	0.03	–	–	0.04
Restionaceae	*Empodisma*	*minus*[§]	–	–	0.01	0.03 (50)	0.04 (33.33)
Asteraceae	*Achillea*	*millefolium*[‡]	0.03	–	–	–	0.03
Asteraceae	***Celmisia***	***pugioniformis***[§]	–	–	–	**0.03**	**0.03**
Asteraceae	***Podolepis***	***robusta***[*]	–	–	–	**0.03**	**0.03**
Ericaceae	***Epacris***	**Unidentified**	–	–	–	**0.03 (100)**	**0.03 (100)**
Caryophyllaceae	*Scleranthus*	*singuliflorus*[§]	–	0.01	0.01	–	0.03
Poaceae	*Deyeuxia*	*carinata*	–	–	0.01	–	0.01
Cyperaceae	*Cyperus*	*eragrostis*[‡]	–	–	0.01	–	0.01
Asteraceae	***Lagenophora***	***stipitata***	–	–	–	**0.01**	**0.01**
Asteraceae	***Brachyscome***	***spathulata*** subsp. ***spathulata***	–	**0.01**	–	–	**0.01**

Table 1 (continued)

Family	Genus	Species	Cool without GA$_3$	Cool with GA$_3$	Warm without GA$_3$	Warm with GA$_3$	Total %
Asteraceae	*Onopordum*	*acanthium*[‡]	–	–	–	**0.01**	**0.01**
Ericaceae	*Epacris*	*petrophylla*	–	–	0.01 (100)	–	0.01 (100)
Onagraceae	*Epilobium*	*sarmentaceum*	0.01	–	–	–	0.01
Onagraceae	*Epilobium*	*gunnianum*	–	–	–	**0.01**	**0.01**
Thymelaeaceae	*Pimelea*	*biflora*[*]	–	–	–	**0.01**	**0.01**
Apiaceae	*Aciphylla*	*glacialis*[§]	–	**0.01**	–	–	**0.01**
Apiaceae	*Oreomyrrhis*	*ciliata*	–	–	–	**0.01**	**0.01**
Crassulaceae	*Crassula*	*sieberiana* s.l.	–	–	–	0.01	0.01
Roseaceae	*Acaena*	*novae-zelandiae*[*]	–	–	–	**0.01**	**0.01**
Total % per treatment			28 (11.17)	24 (6.34)	24 (17.27)	23 (2.81)	100 (10.34)

*More prevalent subalpine.
†Not alpine specific.
‡Exotic weed.
§Also present in the sample site standing vegetation.

Soil warming, germination and total species richness

Contrary to our expectation, soil warming reduced total germination from the alpine soil seed bank (Fig. 1). This result was evident immediately; cumulative data at 3 and 8 weeks post commencement revealed that total germination from soil at cool temperatures was significantly greater than germination from warmed soil ($P < 0.001$, Table 2a). Total germination from cool soil was 99.1% and 44.0% greater than germination from warm soil, at 3 and 8 weeks respectively. Despite reduced germination, total species richness was greater from the warm soil (39 species) than the cool soil (27 species). Although germinant numbers of individual species were often small (Table 1), it is the case that 16 identified species germinated from warm but not cool
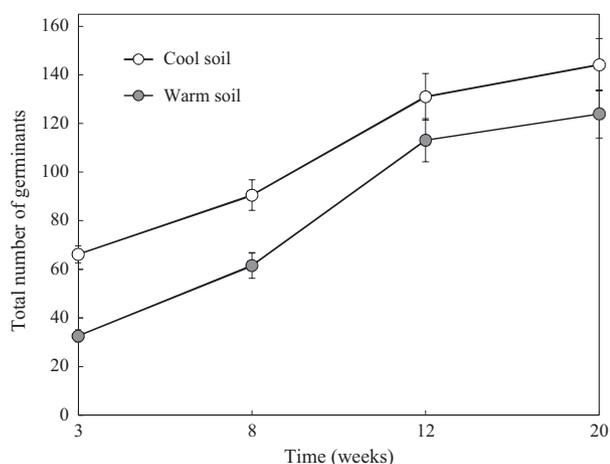

Fig. 1 Total number of germinants from the alpine soil seed bank over time (predicted mean ± standard error), at cool day/night soil temperatures (ca. 19/12 °C), and warm day/night soil temperatures (ca. 23/16 °C).

soil, compared to only seven from cool but not warm soil (Table 1). Given the small number of species, it is perhaps not surprising that these differences are not statistically significant at $P = 0.05$ (Chi square df = 1, $P = 0.14$ and 0.06 respectively).

Germination response to soil warming varied markedly across species. Poaceae seeds made up the bulk of the germinable soil seed bank (63.5% of total germinants), and their germination was significantly reduced by soil warming throughout the entire experiment when compared to germination from soil at cool temperatures ($P < 0.007$, Table 2b; Fig. 2a). In total, warm soil yielded 43.6% fewer Poaceae seedlings than cool soil. In particular, total germination of the most abundant grass, *Rytidosperma nudiflorum*, was 73.6% lower from warm soil compared to cool ($P < 0.001$, Table 2c and Fig. 2b). Soil warming also reduced the rate at which Poaceae seeds germinated. It took more than 8 weeks for 50% of the total Poaceae seedlings to emerge in the warm soil compared to less than 5 weeks in the cool soil (Fig. 2b).

Conversely, soil warming had a positive effect upon germination of some of the less abundant species. Cyperaceae seeds represented 18.9% of the total germinable soil seed bank and germinated significantly more in warm soil than cool throughout the experiment ($P < 0.008$, Table 2d and Fig. 3a). In total, warm soil yielded 48.3% more Cyperaceae seedlings than cool soil (Fig. 3a). This was primarily driven by a 55% increase in total germination of the most abundant sedge, *Carex breviculmis* ($P < 0.001$, Table 2e and Fig. 3b). Similarly, Juncaceae seedlings (99% of which were identified as *Luzula novae-cambriae*), represented 5.65% of the total germinable soil seed bank and increased in total germination by 17.7% in response to soil warming (Tables 1

Table 2 Probability levels (F pr.) from the generalized linear model analyses. Effects of soil temperature, gibberellic acid (GA$_3$) and soil origin (transect), on cumulative germination over time. Significant effects ($P < 0.05$) are in bold

Week	3	8	12	20	Total
(a) Total germination					
Temperature	**<0.001**	**<0.001**	0.181	0.175	0.320
GA$_3$	**<0.001**	0.117	0.972	0.769	0.472
Transect	0.489	0.255	0.059	**0.044**	**0.031**
Temperature × GA$_3$	**0.012**	0.179	0.602	0.453	0.528
Week	**3**	**8**	**12**	**20**	**Total**
(b) Poaceae germination					
Temperature	**<0.001**	**<0.001**	**0.003**	**0.003**	**0.007**
GA$_3$	**0.001**	0.063	0.219	0.389	0.694
Transect	0.918	0.148	**0.021**	**0.017**	**0.012**
Temperature × GA$_3$	**<0.001**	**0.007**	**0.033**	**0.035**	0.054
Week	**3**	**8**	**12**	**20**	**Total**
(c) R. nudiflorum (Poaceae) germination					
Temperature	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
GA$_3$	**0.005**	**<0.001**	**0.005**	**0.011**	0.072
Transect	0.881	0.340	0.491	0.467	0.217
Temperature × GA$_3$	**0.004**	**0.002**	**0.010**	**0.009**	**0.044**
Week	**3**	**8**	**12**	**20**	**Total**
(d) Cyperaceae germination					
Temperature	**0.007**	**<0.001**	**<0.001**	**<0.001**	**0.008**
GA$_3$	0.538	**0.011**	**0.003**	**0.004**	**0.010**
Transect	0.477	0.685	0.177	0.221	0.231
Temperature × GA$_3$	0.423	0.647	0.743	0.952	0.917
Week	**3**	**8**	**12**	**20**	**Total**
(e) C. breviculmis (Cyperaceae) germination					
Temperature	**0.008**	**<0.001**	**<0.001**	**0.001**	**0.001**
GA$_3$	0.675	**0.017**	**0.005**	**0.006**	**0.008**
Transect	0.580	0.692	0.161	0.212	0.291
Temperature × GA$_3$	0.375	0.710	0.815	0.993	0.934
Week	**3**	**8**	**12**	**20**	**Total**
(f) Juncaceae germination					
Temperature	0.083	**0.042**	0.849	0.779	0.907
GA$_3$	0.223	0.463	**0.010**	**0.007**	**0.021**
Transect	0.584	0.859	0.628	0.601	0.237
Temperature × GA$_3$	0.822	0.663	0.295	0.282	0.557

and 2f). *Wahlenbergia ceraceae* (Campanulaceae) seedlings represented just 1.29% of the total germinants but more than tripled in number following soil warming (Table 1). Regardless of soil temperature, germination of Cyperaceae seeds exhibited a lag time before significant germination commenced (Fig. 3a). This lag time (t_{50}, time it took for 50% of the final Cyperaceae germination to be achieved), was, however, reduced from 11+ weeks to 8+ weeks by soil warming (Fig. 3a).

Evidence of physiological dormancy

Of the 45 identified species in the germinable soil seed bank, 17 of them (37.8% of species), representing nine families, only germinated in soil that was treated with GA$_3$ (Table 1). Results suggest that these seeds possessed a non deep or intermediate level of PD that was bypassed by GA$_3$. The exotic weed *Myosotis laxa* (Boraginaceae) germinated exclusively from soil treated with GA$_3$, preferring cool temperatures for germination (Table 1). In contrast, the majority of the species that responded to GA$_3$ germinated in warmed soil (66.7%, Table 1). In particular, total germination of *Rytidosperma nudiflorum* (Poaceae) seeds more than doubled following application of GA$_3$ to warm soil, compared to an increase in just 8% in cool soil (Temperature × GA$_3$ $P < 0.044$, Tables 1 and 2c). In contrast, application of GA$_3$ resulted in significantly fewer *Carex breviculmis* (Cyperaceae, $P < 0.008$, Table 2e), Juncaceae ($P < 0.021$, Table 2f) and *Wahlenbergia ceraceae* (Campanulaceae) seedlings, compared to soil without GA$_3$ (Table 1).

Soil disturbance and germination

As predicted, disturbing the soil significantly boosted germination ($P < 0.001$, Table 3), with 10.3% of the total germinable seed bank germinating following soil disturbance (Table 1, Figs 2 and 3). Consistent with pre disturbance germination, and focusing on trays without GA$_3$, the majority of post disturbance seedlings were Poaceae (38.0%) Cyperaceae (24.2%) and Juncaceae (14.2%) species. Seeds of a further 13 families also germinated (Table 1). Germination response to soil disturbance varied markedly across species. *Cyperus flavidus* (Cyperaceae, Fig. 3c), *Euchiton fordianus* (Asteraceae) and the exotic weed *Acetosella vulgaris* (Polygonaceae) all germinated significantly more in the 10 weeks post disturbance compared to the 20 weeks pre disturbance (99%, 100% and 98% respectively), while other species failed to germinate post disturbance including Fabaceae, Apiaceae and the majority of Asteraceae (Table 1). There were significant interactions between soil warming and disturbance (Table 3), and, in contrast to pre disturbance germination, there were 51.3% more seedlings in the warm than in the cool soil, post disturbance. This was in part due to higher germination of the aforementioned *A. vulgaris* in the warm soil than the cool, post disturbance (Table 1). In stark contrast to Cyperaceae germination pre disturbance, the post disturbance germination of *C. flavidus* (Cyperaceae) was immediate, and greatest in cool soil (Fig. 3c). There were also significant interactions between application of GA$_3$ in week 1 and soil disturbance at week 20 (Table 3); in
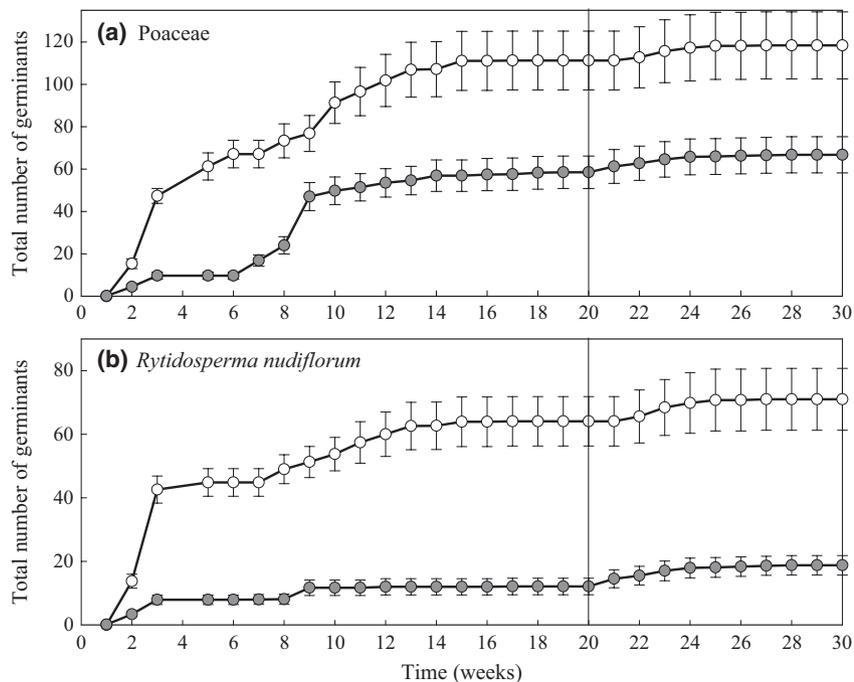
Fig. 2 Number of (a) total Poaceae and (b) *Rytidosperma nudiflorum* (Poaceae) seedlings to emerge from alpine soil, cumulative at each week (mean ± standard error), without the aid of gibberellic acid, at cool day/night soil temperatures (ca. 19/12 °C, open circles), and warm day/night soil temperatures (ca. 23/16 °C, closed circles). The vertical line indicates when the soil was disturbed.

particular, post disturbance germination of *R. nudiflorum* (Poaceae) was greater in trays that did not receive GA_3 in week 1 compared to trays that did, regardless of temperature (Table 1).

Comparison of in situ standing vegetation with germinable soil seed bank

The standing vegetation within all the quadrats from which soil samples were taken consisted of 43 species across 21 families (Table S1). In total, 18 species were found in both the standing vegetation and the germinable soil seed bank (Table 1 and Fig. 4a), meaning that almost 42% of species in the standing vegetation was represented in the soil. Of these 18 species, soil warming increased germination of 11 and soil disturbance promoted germination of seven, but only five species revealed evidence of PD in response to application of GA_3. At the individual quadrat level, the mean number of species shared between the standing vegetation and soil seed bank (±standard error), was considerably lower than that at the site level: only 2.3 ± 0.4 (Fig. 4b). Mean species richness per quadrat was also lower than the total species richness: 9 ± 0.6 species in the standing vegetation and 13 ± 0.8 species in the soil seed bank (Fig. 4b). Given this variation in species composition among quadrats, it is to be expected that the qualitative (SJ) and quantitative (SR) indices

(means ± standard error), revealed low overall similarities between the standing vegetation and the soil seed bank: SJ = 0.11 ± 0.02, SR = 0.03 ± 0.02). Notably, three of the four most prevalent species in the standing vegetation were *Poa* grasses (*P. hiemata*, *P. costiniana* and *P. fawcettiae*), which were not represented at all in the germinable soil seed bank.

Discussion

To the best of our knowledge, this is the first investigation into the effect of soil warming on germination from a persistent, alpine soil seed bank. Contrary to expectations, soil warming leads to a reduction in overall germination. Closer examination of the data reveals that germination response to soil temperature was species specific and, for some species, varied with time and soil disturbance. This study is also the first to investigate the prevalence of seed physiological dormancy (PD) within an alpine soil seed bank. Results suggest that seeds of a significant proportion of species in the germinable soil seed bank possess PD mechanisms; therefore, the effects of climate change upon the dormancy status of seeds in the soil should not be ignored. Lastly, results suggest that germination of a range of alpine species is intrinsically linked to soil disturbance. We will discuss these main findings in relation to the role of the alpine soil seed bank in species shift and community composition in the future.
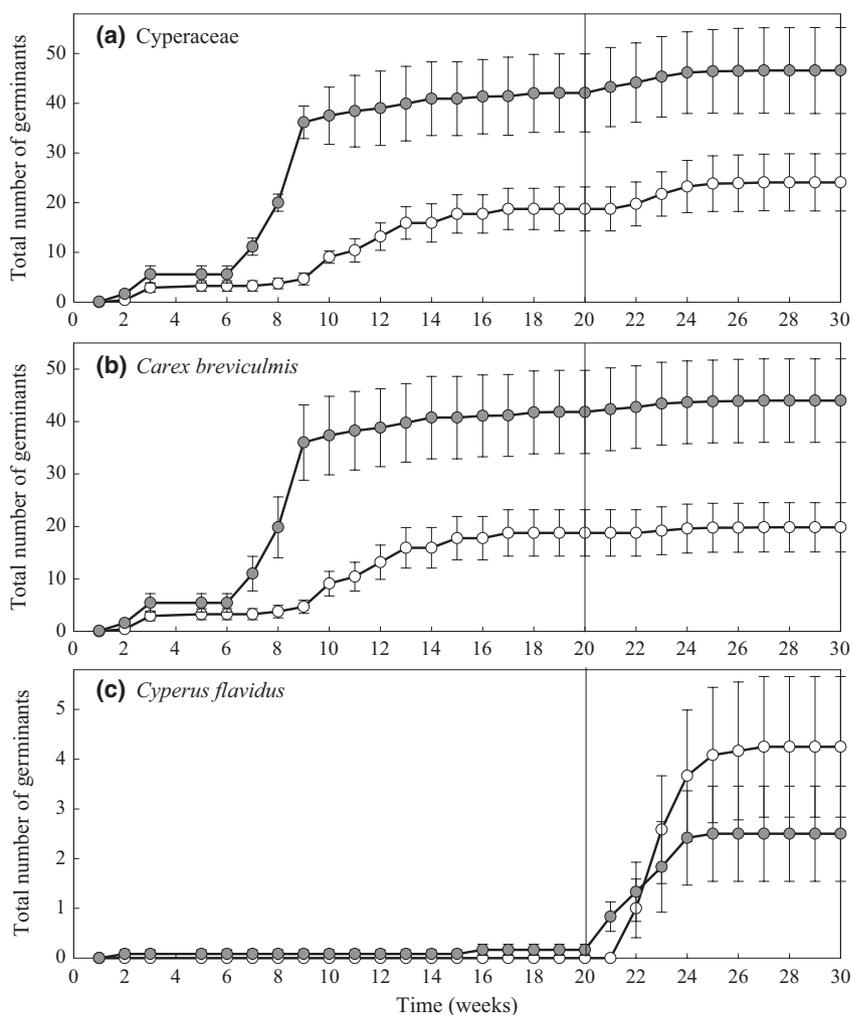
Fig. 3 Number of (a) total Cyperaceae, (b) *Carex breviculmis* and (c) *Cyperus flavidus* (Cyperaceae) seedlings to emerge from alpine soil, cumulative at each week (mean ± standard error), without the aid of gibberellic acid, at cool day/night soil temperatures (ca. 19/12 °C, open circles), and warm day/night soil temperatures (ca. 23/16 °C, closed circles). The vertical line indicates when the soil was disturbed.

Germination response to soil warming was species specific

We expected soil warming to increase overall germination from the alpine soil seed bank, as reported for boreal wetland (Hogenbirk & Wein, 1992), subalpine meadow (Harte & Shaw, 1995), temperate forest (Thompson & Naeem, 1996) and Alaskan tundra (Hobbie & Chapin III, 1998) soil seed banks. Instead, we saw an overall reduction in germination following soil warming, which was the result of the significant response of a single Poaceae species; *Rytidosperma nudiflorum*. Grasses are generally predicted to become more productive under a warmer Arctic tundra climate (Dormann & Woodin, 2002; Walker *et al.*, 2006). Poaceae seed germination success is predicted to be favoured by warming and contribute to the expected expansion of

grasses in tundra (Shevtsova *et al.*, 2009). In part, our results may be explained by reduced viability of *R. nudiflorum* seeds in the warmed soil, because imbibed seeds are understood to age more rapidly as temperatures increase (Ellis & Roberts, 1980). Alternatively, current alpine soil temperatures may already be close to upper limits for germination of *R. nudiflorum*. Our results may contradict previous work due to the lesser degree of warming investigated in some previous studies (e.g. Harte & Shaw, 1995 and Hobbie & Chapin, 1998). However, similar and greater temperature increases have initiated increased germination of alpine seeds *ex situ* (Graae *et al.*, 2008; Milbau *et al.*, 2009; Mondoni *et al.*, 2012). The lack of *Poa* germination from the soil seed bank in this grass dominated community may indicate that these species are part of the transient soil seed bank or have dormancy mechanisms that we did not alleviate.

Table 3 Probability levels (F pr.) from the split-plot analyses of the effect of soil temperature, gibberellic acid (GA₃) and soil disturbance on cumulative germination between week 20 and 30; Total germination and that of Poaceae, Cyperaceae and Juncaceae seeds. Significant effects ($P < 0.05$) are in bold

	Total	Poaceae	Cyperaceae	Juncaceae
Temperature	ns	**<0.001**	**<0.001**	**<0.001**
GA₃	ns	ns	**<0.001**	**<0.001**
Disturbance	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Temp × GA₃	ns	**<0.01**	ns	ns
Temp × Disturbance	**0.007**	**0.004**	**<0.001**	0.198
GA₃ × Disturbance	**0.001**	**<0.001**	0.282	**0.008**

In contrast, seeds less abundant in the germinable soil seed bank, including those of Cyperaceae, Juncaceae and Campanulaceae species, appeared better suited to germination at warmer temperatures. It is worth noting that Cyperaceae species are well known for increased germination at higher temperatures (Schutz, 2000). Boreal wetland soil collected in Alberta, Canada also yielded more Cyperaceae seedlings at high temperatures (30/15 °C day/night) compared to moderate temperatures (20/10 °C, Hogenbirk & Wein, 1992). *In situ*, germination of non dormant seeds is thought to occur soon after snow melt in early spring (Billings & Mooney, 1968), which is optimal given the short alpine growing season. Premature melting of snow and ice is a well-publicized consequence of climate change (IPCC, 2007), with seedling establishment in the Subarctic expected to improve in the future due to longer (earlier) growing seasons and accelerated germination rates (Milbau *et al.*, 2009). However, we saw reduced germination rate in response to soil warming across total Poaceae germination. Results suggest that longer growth seasons may not bestow the expected advantages for all plant recruitment via the soil seed bank when in combination with soil warming (Galen & Stanton, 1999). Delayed germination

may result in greater winter mortality if seedlings have not reached the optimal size for overwintering (Shimono & Kudo, 2003). In contrast, soil warming reduced the lag phase that was exhibited by Cyperaceae seedling emergence. *In situ*, a lag phase following spring warming of soil and 'late' germination of Cyperaceae seeds is well documented (Leck & Schutz, 2005), and currently appears to restrict the range of many sedges (Grime *et al.*, 1988). We conclude that changes in recruitment via the alpine soil seed bank in response to increased soil temperatures will alter the relative abundance of certain tall alpine herbfield taxa. Warming may also facilitate changes in species dominance, particularly if current dominant *Poa* species do not persist in the soil seed bank, and support new 'stable states' within the native vegetation.

Physiological dormancy was prevalent among species in the soil seed bank

Seeds of species belonging to a wide range of families, including Poaceae, Boraginaceae, Asteraceae, Apiaceae, Crassulaceae and Rosaceae species, appear to possess PD mechanisms. Seeds that responded to the application of GA₃ may not have received sufficient dormancy alleviation *in situ* to enable germination without GA₃, or may have cycled back into dormancy as a result of inadequate germination cues (Baskin & Baskin, 1989). Dormancy cycling in the soil seed bank is common and can contribute to the persistence of seeds through time (Fenner & Thompson, 2005; Mennan & Zandstra, 2006). We saw evidence of more species having PD in warm than cool soil. One explanation for this is that warm stratification altered sensitivity to GA₃ through changes in seed dormancy status (Derkx & Karssen, 1993), raising questions regarding the effects of soil temperature on dormancy status of seeds in the soil seed bank. For example, *R. nudiflorum* (Poaceae) seeds appeared to lose dormancy over time in the warm soil, resulting in post disturbance germination that, in the presence of GA₃,
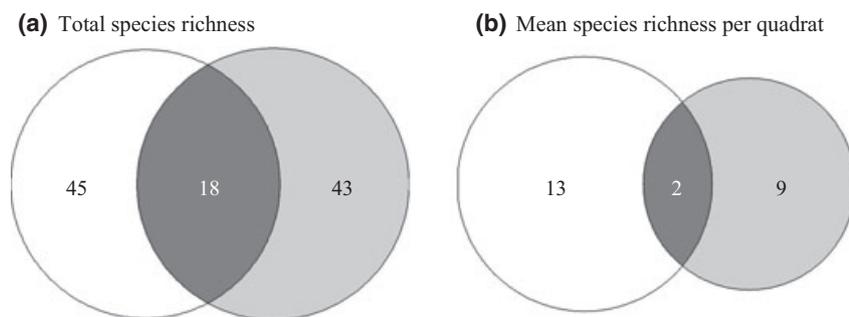
(a) Total species richness

(b) Mean species richness per quadrat

Fig. 4 Species similarity between the *in situ* standing vegetation and the germinable soil seed bank. The total species richness across all quadrats (a) in the soil seed bank (white), standing vegetation (light grey) and the overlap (dark grey); and the rounded mean species richness per quadrat (b) in the soil seed bank (white), standing vegetation (light grey) and the overlap (dark grey).

happened much earlier in the experiment. It is possible, even likely, that dormancy is more common than even our data imply. Deep PD was the most abundant dormancy type among 28 alpine species investigated in Austria (Schwienbacher *et al.*, 2011), but cannot be bypassed by GA$_3$ alone (Baskin & Baskin, 2004), therefore, would not have been detected in our study. Finally, despite increased diversity, our results suggest that GA$_3$ at the concentration, volume and/or temperatures used was detrimental to the germination of some species. Seeds in the persistent soil seed bank, which will not only have aged since dispersal but may also have altered in their germination requirements and dormancy status, are likely to exhibit a different germination response to changes in temperature compared to freshly dispersed seeds from the same plant/species. *In situ*, PD currently plays a key role in optimizing alpine germination success by controlling the timing of germination (Simons & Johnston, 2000; Donohue, 2005). Conservative germination strategies that involve dormancy mechanisms may optimize seedling establishment under current alpine climes, but changes in soil temperature are likely to affect the dormancy status of PD seeds in the soil seed bank and will alter and/or disrupt these strategies. For example, climate warming is expected to lead to a shift from spring to autumn emergence of alpine plants, driven primarily by seed dormancy status and resulting in major implications for species currently adapted to emergence in spring (Mondoni *et al.*, 2012).

Soil disturbance promoted germination

Soil seed banks are particularly important in the re-establishment of vegetation after disturbance (Bakker *et al.*, 1996; Funes *et al.*, 2001; Fenner & Thompson, 2005). Whether the post disturbance germination that we saw was in response to changes in temperature, humidity, moisture or light availability associated with turning the soil over, or physiological changes within the seeds themselves over time, is not clear. It is possible that a stratification effect upon seeds and subsequent alleviation of deep PD contributed to the significant post disturbance germination from warm soil, in which case increased disturbance events (Worboys & Good, 2011) may boost recruitment levels of species better suited to germination and establishment under new climates. Results suggest that more than half of the species diversity within the soil seed bank came from plants that have perished and/or are growing outside the sample site. However, we found no evidence of any subalpine species in the soil and only very low numbers of exotic weed seeds. Results suggest that the soil seed bank will not facilitate the increase in the range of subalpine and exotic species expected under climate change (Pickering

et al., 2008), however, nor will temperature increases compromise recruitment via the soil seed bank during important revegetation periods.

Future directions and implications for alpine management

Important questions remain regarding alpine seed set, viability, longevity, dormancy status and germination success with predicted changes in soil temperature. The expected increase in alpine seed production and quality with warming (Arft *et al.*, 1999) may counteract any reductions in germinability and seedling establishment. In *ex situ* storage, the controlled ageing test (CAT, Davies & Probert, 2004) has predicted that alpine seeds are shorter lived than those of species originating in warm, dry climates (Probert *et al.*, 2009). A possible correlation between CAT results and field persistence for alpine seeds, as demonstrated for European and Australian weed seeds (Long *et al.*, 2008), may aid prediction of seed longevity in alpine soil seed banks both now and in the future. With increasing evidence of PD among alpine seeds, the impact of climate change on alpine germination strategies warrants further study. For example, changes in seed dormancy status which affect the timing of germination may subject alpine seedlings to seasonal conditions to which they are not adapted (Mondoni *et al.*, 2012). Changes in both temperature and soil moisture on early seedling growth are likely to be more important for seedling establishment than the effect of either variable acting alone (Walck *et al.*, 2011 and studies reviewed therein). Finally, we do not know the mechanistic impacts of climate change on the soil seed bank beneath other threatened alpine communities such as short alpine herbfield, feldmark and sod tussock grassland.

It is recognized that alpine plant communities will alter under climate change in terms of species composition, distribution and range, and regeneration via soil seed banks will undoubtedly play a key role in this evolution. Alpine soil surface temperatures have been found to deviate strongly from air temperatures, resulting in thermal microhabitat mosaics across mountain terrain (Scherrer & Körner, 2010). With this in mind, temperature effects upon seed germination are likely to be highly variable, localized and dependent on topography. However, our results demonstrate that altered dormancy status, germination requirements and thus germination of seeds in the soil seed bank can be expected. Understanding the impacts of climate change on soil seed bank dynamics will not only facilitate accurate predictions of species distributions and risk of extinction but also highlight species best suited to alpine restoration, ensuring establishment and longevity of a self-sustaining suite of native species.

Acknowledgements

References

Archibold OW (1984) A comparison of seed reserves in arctic, subarctic and alpine soils. *Canadian Field-Naturalist*, **98**, 337–344.

Arft AM, Walker MD, Gurevitch J et al. (1999) Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological Monographs*, **69**, 491–511.

Arroyo MTK, Cavieres LA, Castor C, Humana AM (1999) Persistent soil seed bank and standing vegetation at a high alpine site in the central Chilean Andes. *Oecologia*, **119**, 126–132.

Bakker JP, Poschlod P, Strykstra RJ, Bekker RM, Thompson K (1996) Seed banks and seed dispersal: important topics in restoration ecology. *Royal Botanical Society of the Netherlands*, **45**, 461–490.

Baskin C, Baskin J (1989) Physiology of dormancy and germination in relation to seed bank ecology. In: *Ecology of Soil Seed Banks* (eds Leck MA, Parker VT, Simpson RL), pp. 53–66. Academic Press Inc, San Diego.

Baskin J, Baskin C (2004) A classification system for seed dormancy. *Seed Science Research*, **14**, 1–16.

Bekker RM, Bakker JP, Grandin U et al. (1998) Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology*, **12**, 834–842.

Bell DT (1999) The process of germination in Australian species. *Australian Journal of Botany*, **47**, 475–517.

Benech-Arnold RL, Sanchez RA, Forcella F, Kruk BC, Ghersa CM (2000) Environmental control of dormancy in weed seed banks in soil. *Field Crops Research*, **67**, 105–122.

Beniston M (2003) Climate change in mountain regions: a review of possible impacts. *Climate Change*, **59**, 5–31.

Billings WD, Mooney HA (1968) Ecology of arctic and alpine plants. *Biological Reviews of the Cambridge Philosophical Society*, **43**, 481–529.

Bliss LC (1971) Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics*, **2**, 405–438.

Brown JS, Venable DL (1986) Evolutionary ecology of seed-bank annuals in temporally varying environments. *The American Naturalist*, **127**, 31–47.

Bueno CG, Reine R, Alados CL, Gomez-Garcia D (2011) Effects of large wild boar disturbances on alpine soil seed banks. *Basic and Applied Ecology*, **12**, 125–133.

Bureau of Meteorology (2010) Climate statistics at Australian alpine locations between 1966 and 2009. Available at: http://www.bom.gov.au/climate/averages/tables/cw_071003.shtml (accessed January 2010)

Cavieres LA, Arroyo MTK (2001) Persistent soil seed banks in *Phacelia secunda*: experimental detection of variation along an altitudinal gradient in the Andes of central Chile (33°S). *Journal of Ecology*, **89**, 31–39.

Cerabolini B, Ceriani RM, Caccianiga M, De Andreis R, Raimondi B (2003) Seed size, shape and persistence in soil: a test on Italian flora from Alps to Mediterranean coasts. *Seed Science Research*, **13**, 75–85.

Chambers JC (1995) Disturbance, life history strategies and seed fates in alpine herbfield communities. *American Journal of Botany*, **82**, 421–433.

Costin A, Gray M, Totterdell C, Wimbush D (2000) *Kosciuszko Alpine Flora*. CSIRO Publishing, Australia.

Davies H, Probert R (2004) *Protocol for Comparative Seed Longevity Testing*. The Millennium Seed Bank, Kew, West Sussex, UK.

Derkx MPM, Karssen CM (1993) Effects of light and temperature on seed dormancy and gibberellin-stimulated germination in *Arabidopsis thaliana*: studies with gibberellin-deficient and -insensitive mutants. *Physiologia Plantarum*, **89**, 360–368.

Diaz HF, Bradley RS (1997) Temperature variations during the last century at high elevation sites. *Climate Change*, **36**, 253–279.

Diemer M, Prock S (1993) Estimates of alpine seed bank size in 2 central-European and 1 Scandinavian sub-arctic plant communities. *Arctic and Alpine Research*, **25**, 194–200.

Donohue K (2005) Seeds and seasons: interpreting germination timing in the field. *Seed Science Research*, **15**, 175–187.

Dormann CF, Woodin SJ (2002) Climate change in the Arctic: using plant functional types in a meta-analyisis of field experiments. *Functional Ecology*, **16**, 4–17.

Dullinger S, Hulber K (2011) Experimental evaluation of seed limitation in Alpine snowbed plants. *PLoS ONE*, **6**, e21537. doi: 10.1371/journal.pone.0021537

Ellis RH, Roberts EH (1980) The influence of temperature and moisture on seed viability period in barley (*Hordeum distichum* L.). *Annals of Botany*, **45**, 31–37.

Erschbamer B, Niederfriniger S, Ruth Winkler E (2008) Colonization processes on a central Alpine glacier foreland. *Journal of Vegetation Science*, **19**, 855–862.

Fenner M, Thompson K (2005) *The Ecology of Seeds*. Cambridge University Press, Cambridge.

Forbis TA (2003) Seedling demography in an alpine ecosystem. *American Journal of Botany*, **90**, 1197–1206.

Funes G, Basconcelo S, Diaz S, Cabido M (2001) Edaphic patchiness influences grassland regeneration from the soil seed-bank in mountain grasslands of central Argentina. *Austral Ecology*, **26**, 205–212.

Galen C, Stanton ML (1999) Seedling establishment in alpine Buttercups under experimental manipulations of growing season length. *Ecology*, **80**, 2033–2044.

Graae BJ, Alsos IG, Ejrnaes R (2008) The impact of temperature regimes on development, dormancy breaking and germination of dwarf shrub seeds from arctic, alpine and boreal sites. *Plant Ecology*, **198**, 275–284.

Green K, Pickering C (2009) The decline of snowpatches in the Snowy Mountains of Australia: importance of climate warming, variable snow and wind. *Arctic, Antarctic and Alpine Research*, **41**, 212–218.

Grime JP, Hodgeson JG, Hunt R (1988) *Comparative Plant Ecology: A functional Approach to Common British Plants*. Unwin Hyman, London.

Harte J, Shaw R (1995) Shifting dominance within a montane vegetation community: results of a climate-warming experiment. *Science*, **267**, 876–880.

Hennessy K, Whetton P, Smith I, Bathols J, Hutchinson M, Sharples J (2003) *The Impact of Climate Change on Snow Conditions in Mainland Australia*. CSIRO Publishing, Aspendale, VIC, Australia.

Hobbie SE, Chapin FS III (1998) An experimental test of limits to tree establishment in Arctic tundra. *Journal of Ecology*, **86**, 449–461.

Hogenbirk JC, Wein RW (1992) Temperature effects on seedling emergence from boreal wetland soils: implications for climate change. *Aquatic Botany*, **42**, 361–373.

IPCC (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group 1 to the Forth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.

Jongman RHG, ter Braak CJF, van Tongeren OFR (1987) *Data Analysis in Community and Landscape Ecology*. Pudoc, Wageningen, The Netherlands.

Klug-Pumpel B, Scharfetter-Lehrl G (2008) Soil diaspore reserves above the timberline in the Austrian Alps. *Flora*, **203**, 292–303.

Körner C (2003) Alpine seed banks and natural recruitment. In: *Alpine Plant Life; Functional Plant Ecology of High Mountain Ecosystems*, 2nd edn (ed. Körner C), pp. 274–278. Springer-Verlag, Berlin, Heidelberg, New York.

Kullman L (2004) The changing face of the alpine world. *Global Change Newsletter*, **57**, 12–14.

Leck MA, Schutz W (2005) Regeneration of Cyperaceae with particular reference to seed ecology and seed banks. *Perspectives in Plant Ecology, Evolution and Systematics*, **7**, 95–133.

Levin DA (1990) The seed bank as a source of genetic novelty in plants. *The American Naturalist*, **135**, 563–572.

Li Q, Fang H, Cai Q (2011) Persistent soil seed banks along altitudinal gradients in the Qilian Mountains in China and their significance for conservation management. *African Journal of Agricultural Research*, **6**, 2329–2340.

Long RL, Panetta FD, Steadman KJ, Probert R, Bekker RM, Brooks S, Adkins SW (2008) Seed persistence in the field may be predicted by laboratory-controlled aging. *Weed Science*, **56**, 523–528.

Marcante S, Schwienbacher E, Erschbamer B (2009) Genesis of a soil seed bank on a primary succession in the central Alps (Otztal, Austria). *Flora*, **204**, 434–444.

Mattana E, Daws MI, Bacchetta G (2009) Seed dormancy and germination ecology of *Lamyropsis microcephala*: a mountain endemic species of Sardinia (Italy). *Seed Science and Technology*, **37**, 491–497.

McGraw JB, Vavrek MC (1989) The role of buried viable seeds in Arctic and Alpine plant communities. In: *Ecology of Soil Seed Banks* (eds Leck MA, Parker VT, Simpson RL), pp. 91–106. Academic Press Inc, San Diego.

Mennan H, Zandstra BH (2006) The effects of depth and duration of seed burial on viability, dormancy, germination and emergence of Ivyleaf Speedwell (*Veronica huderifolia*). *Weed Technology*, **20**, 438–444.

Milbau A, Graae BJ, Shevtsova A, Nijs I (2009) Effects of a warmer climate on seed germination in the subarctic. *Annals of Botany*, **104**, 287–296. doi: 10.1093/aob/mcp117

Mondoni A, Daws MI, Belotti J, Rossi G (2009) Germination requirements of the alpine endemic *Silene elisabethae* Jan: effects of cold stratification, light and GA₃. *Seed Science and Technology*, **37**, 79–87.

Mondoni A, Rossi G, Orsenigo S, Probert RJ (2012) Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany*, **110**, 155–164. doi: 10.1093/aob/mcs097

Ooi MJ, Auld TD, Denham AJ (2009) Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. *Global Change Biology*, **15**, 2375–2386.

Philippi T (1993) Bet-hedging germination of desert annuals: beyond the first year. *The American Naturalist*, **142**, 474–487.

Pickering C, Hill W, Green K (2008) Vascular plant diversity and climate change in the alpine zone of the Snowy Mountains, Australia. *Biodiversity and Conservation*, **17**, 1627–1644.

PlantNET (2012) The plant information network system of the royal botanic gardens and domain trust. Version 2.0. Available at: www.plantnet.rbgsyd.nsw.gov.au (accessed 2 January 2012)

Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of *ex situ* seed longevity: a comparative study on 195 species. *Annals of Botany*, **104**, 57–69. doi: 10.1093/aob/mcp082

Sayers RL, Ward RT (1966) Germination responses in alpine species. *Botanical Gazette*, **127**, 11–16.

Scherrer D, Körner C (2010) Infra-red thermometry of alpine landscapes challenges climatic warming projections. *Global Change Biology*, **16**, 2602–2613.

Schlag RN, Erschbamer B (2000) Germination and establishment of seedlings on a glacier foreland in the central Alps, Austria. *Arctic, Antarctic and Alpine Research*, **32**, 270–277.

Schutz W (2000) Ecology of seed dormancy and germination in sedges (*Carex*). *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 67–89.

Schwienbacher E, Navarro-Cano JA, Neuner G, Erschbamer B (2011) Seed dormancy in alpine species. *Flora*, **206**, 845–856.

Shevtsova A, Graae BJ, Jochum T, Milbau A, Kockelbergh F, Beyenss L, Nijs I (2009) Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. *Global Change Biology*, **15**, 2662–2680.

Shimono Y, Kudo G (2003) Intraspecific variations in seedling emergence and survival of *Potentilla matsumura* (Rasaceae) between alpine fellfield and snowbed habitats. *Annals of Botany*, **91**, 21–29.

Simons AM, Johnston MO (2000) Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *American Journal of Botany*, **87**, 124–132.

Simons AM, Johnston MO (2006) Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet-hedging. *Evolution*, **60**, 2280–2292.

Thompson K, Grime JP (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology*, **67**, 893–921.

Thompson LJ, Naeem S (1996) The effects of soil warming on plant recruitment. *Plant and Soil*, **182**, 339–343.

Thompson K, Band SR, Hodgson JG (1993) Seed size and shape predict persistence in soil. *Functional Ecology*, **7**, 236–241.

Turner SR, Merritt DJ (2009) Seed germination and dormancy. In: *Plant Germplasm Conservation in Australia: Strategies and Guidelines for Developing, Managing an Utilizing ex situ collections* (eds Offord CA, Meagher PF), pp. 87–108. Australian Network for Plant Conservation Inc., Canberra.

Venable DL, Brown JS (1988) The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risk in variable environments. *The American Naturalist*, **131**, 360–384.

Venn SE, Morgan JW (2009) Germination characteristics of Mountain Celery *Aciphylla glacialis* (F. Muell.) Benth. (Apiaceae). *The Victorian Naturalist*, **126**, 4–12.

Venn S, Morgan J (2010) Soil Seedbank composition and dynamics across alpine summits in south-eastern Australia. *Australian Journal of Botany*, **58**, 349–362.

Vleeshouwers LM, Bouwmeester HJ (2001) A simulation model for seasonal changes in dormancy and germination of weed seeds. *Seed Science Research*, **11**, 77–92.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology*, **83**, 1031–1037.

Walck J, Baskin J, Baskin C, Hidayati S (2005) Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. *Seed Science Research*, **15**, 189–196.

Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P (2011) Climate change and plant regeneration from seed. *Global Change Biology*, **17**, 2145–2161.

Walker MD, Wahren CH, Hollister RD *et al*. (2006) Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences*, **103**, 1342–1346.

Welling P, Laine K (2000) Characteristics of the seedling flora in alpine vegetation, subarctic Finland, II. Floristic similarity between seedling flora and mature vegetation. *Annales Botanici Fennici*, **37**, 133–147.

Welling P, Tolvanen A, Laine K (2004) The alpine soil seed bank in relation to field seedlings and standing vegetation in subarctic Finland. *Arctic, Antarctic and Alpine Research*, **36**, 229–238.

Whitaker D, Williams ER, John JA (2009) *CycDesigN 4.0: A Package for the Computer Generation of Experimental Designs*. CycSoftware Ltd., Naseby, New Zealand.

Worboys GL, Good R (2011) *Caring for Our Australian Alps Catchments*. Summary Report for Policy Makers, The Australian Government, Canberra.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. *In situ* standing vegetation. Species found in the standing vegetation across all 12 *in situ* quadrats, including the number of quadrats they were found in and the mean percentage cover within those quadrats. Listed by percentage cover (largest to smallest). A total of 43 species representing 21 families and 31 genera.