Lowland plant migrations into alpine ecosystems amplify soil carbon loss under climate warming

Tom W. N. Walker^{1 *}, Konstantin Gavazov^{2,3,4}, Thomas Guillaume^{3,4,5}, Thibault Lambert⁶, Pierre Mariotte^{3,4,7}, Devin Routh¹, Constant Signarbieux^{3,4}, Sebastián Block^{1,8}, Tamara Münkemüller⁹, Hanna Nomoto¹, Thomas W. Crowther¹, Andreas Richter¹⁰, Alexandre Buttler^{3,4} & Jake M. Alexander¹

¹ Institute of Integrative Biology, ETH Zürich, 8092 Zürich, Switzerland.

² Climate Impacts Research Centre, Department of Ecology and Environmental Sciences, Umeå Universitet, 98107 Abisko, Sweden

³ Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), 1015 Lausanne, Switzerland.

⁴ École Polytechnique Fédérale de Lausanne EPFL, School of Architecture, Civil and Environmental Engineering ENAC, Laboratory of Ecological Systems ECOS and Plant Ecology Research Laboratory PERL, 1015 Lausanne, Switzerland.

⁵ Field-Crop Systems & Plant Nutrition, Agroscope, 1260 Nyon, Switzerland.

⁶ Faculty of Geosciences & the Environment, Université de Lausanne, 1015 Lausanne, Switzerland.

⁷ Grazing Systems, Agroscope, 1260 Nyon, Switzerland.

⁸ Department of Ecology & Evolutionary Biology, Princeton University, 08544 Princeton, USA.

⁹ Uni. Grenoble Alpes, CNRS, Uni. Savoie Mont Blanc, LECA, Laboratoire d'Ecologie Alpine, F-38000, Grenoble, France.

¹⁰ Centre of Microbiology & Environmental Systems, Division of Terrestrial Ecosystem Research, University of Vienna, 1090 Vienna, Austria.

¹¹ International Institute for Applied Systems Analysis, Ecosystem Services and Management Program, 2361 Laxenburg, Austria

* Correspondence to: thomas.walker@usys.ethz.ch.

25 **Abstract**

26 Climate warming is releasing carbon from soils around the world^{1–3}, constituting a positive climate
 27 feedback. Warming is also causing species to expand their ranges into new ecosystems^{4–9}. Yet, in
 28 most ecosystems, whether range expanding species will amplify or buffer expected soil carbon loss
 29 is unknown¹⁰. Here we used alpine grasslands as a model system to determine whether the
 30 establishment of herbaceous lowland plants in alpine ecosystems influences short-term soil carbon
 31 storage under warming. We found that warming (< 1 year) led to negligible alpine soil carbon loss,
 32 but its effects became significant and 52% ± 31% (mean ± 95% CIs) larger after lowland plants were
 33 introduced at low density into the ecosystem. We present evidence that soil carbon loss likely
 34 occurred *via* lowland plants increasing rates of root exudation, soil microbial respiration and CO₂
 35 release. Our findings suggest that warming-induced range expansions of herbaceous plants may
 36 yield a rapid positive climate feedback in this system, and that plant range expansions among
 37 herbaceous communities may be an overlooked mediator of warming effects on carbon dynamics.

38 Introduction

39 Climate warming is accelerating the release of soil carbon to the atmosphere^{1–3}, creating a positive
 40 climate feedback. At the same time, warming is driving uphill and poleward range expansions of
 41 native plant species around the globe^{4–9}. Yet while warming and plant range expansions are now
 42 co-occurring in many terrestrial ecosystems, the impact of range expanding plants on soil carbon
 43 storage under warming remains poorly understood¹⁰. This is important because plant species
 44 adapted to different temperatures can have contrasting effects on carbon cycle processes¹¹, raising
 45 the potential for migrating species to have qualitatively different impacts on carbon dynamics from
 46 resident plants. For example, studies of non-native species invasions have revealed that the arrival
 47 of novel species in a community can alter carbon cycling in recipient ecosystems^{12,13}, while woody
 48 plant expansions into treeless Arctic ecosystems can reverse the direction of carbon cycle feedbacks
 49 to climate warming^{14,15}. However, the majority of warming-induced range expansions do not involve
 50 non-native species or novel growth forms, but occur among native communities of similar species
 51 (e.g. ref⁹). In particular, we know remarkably little about how migrations of herbaceous plants into
 52 predominantly herbaceous ecosystems will affect soil carbon stocks, and so the magnitude and
 53 direction of resulting climate feedbacks are unknown.

54
 55 Alpine ecosystems occur in the uppermost elevations of the planet as cool islands within warmer
 56 landscapes that are bounded by steep gradients in temperature¹⁶. They are experiencing both rapid
 57 temperature change¹⁷ and faster-than-average species immigration¹⁸, the latter of which mainly
 58 involves an influx of herbaceous warm-adapted species from lowlands into herbaceous cold-adapted
 59 communities at higher elevations^{7–9}. Coupled to this, alpine ecosystems are globally relevant
 60 reservoirs of soil carbon and hold more than 90% of their total ecosystem carbon in the soil^{1–3,19,20}.
 61 As such, alpine ecosystems are not only themselves important for climate feedbacks, but are also
 62 sentinels for climate warming impacts on other terrestrial ecosystems. Here, we used alpine
 63 ecosystems as a model system to determine how the establishment of range expanding native plant
 64 species affects soil carbon storage in warming ecosystems. We first used two whole-community
 65 transplant experiments in different alpine regions (western/central Swiss Alps) to establish the
 66 effects of simulated warming and lowland plant arrival on alpine soil carbon loss. Second, we used

these experiments plus glasshouse and laboratory studies to elucidate potential mechanisms through which lowland plants influence the alpine soil carbon cycle, testing the hypothesis that they act *via* changes to root exudation^{21,22}.

Figure 1 | Warming and lowland plant effects on alpine soil carbon loss. Mean $\pm$ SE soil carbon content (mg C g⁻¹ dry mass) in alpine turfs exposed to warming (W; light grey), warming plus lowland plants (WL; dark grey) or an ambient control (C; white). Data are displayed for two experiments in the western (left) and central (right) Alps, with letters indicating treatment differences (LMEs; N = 58).

Results & Discussion

Lowland plants amplify warming-induced soil carbon loss

We exploited two independent field experiments in which alpine plant communities plus rooting zone soil were transplanted from high to low elevation to simulate warming, or back into a high elevation site as a negative control (Supplementary Fig. S1). Half of the alpine communities at low elevation were planted with seedlings of local lowland plant species to simulate the arrival of lowland plants in the ecosystem. Remaining communities at low elevation and all control communities at high elevation were instead subjected to a planting disturbance control (see Methods for a full account of control treatments and validation exercises). We found that warming of less than one year caused a small decline in alpine soil carbon content, but in both regions this effect was stronger and only became statistically significant when lowland plants were also present (Fig. 1; Table S1; $P < 0.001$). Indeed, considering field data from both experiments together, we estimate that the arrival of lowland plants in the ecosystem amplified warming-induced soil carbon loss by $52\% \pm 31\%$ (mean $\pm$ 95%

confidence intervals) per °C relative to warming alone (Supplementary Fig. S3; $P = 0.001$). These findings collectively show that once lowland plants establish in alpine communities they can amplify warming-induced soil carbon loss. We thus suggest that uphill migrations of lowland plants, which are already globally common^{8,18}, have a hitherto unrecognised bearing over alpine soil carbon storage and could ultimately yield a positive climate feedback.

Lowland and alpine plants have distinct effects on soil microbial respiration

Soil carbon loss occurs due to the activity of soil microorganisms, which break down soil organic matter and release a portion as CO₂ through respiration²³. We therefore hypothesised that the observed impact of lowland plants on alpine soil carbon loss was mediated *via* lowland plant effects on soil microbial respiration. To test this, we performed a glasshouse experiment in which we grew lowland plants (*Bromus erectus*, *Salvia pratensis*, *Medicago lupulina*) or alpine plants (*Phleum alpinum*, *Anthyllis vulneraria* subsp. *alpestris*, *Plantago alpina*) in alpine soil collected from the western Alps region. We found that lowland plants accelerated soil microbial respiration relative to alpine plants (Fig. 2a; treatment × time: LR = 147.99, $P < 0.001$). Accelerated microbial activity was also reflected in spectral analyses of soil pore water, which showed that soil under lowland plants contained more dissolved organic matter (Fig. 2d,e; Table S2; a_{350} : $P < 0.001$, F_{tot} : $P < 0.001$) with a higher proportion of microbially-derived products (Fig. 2f; Table S2; FI; $P = 0.002$) than soil under alpine plants. Importantly, microbial respiration was enhanced despite no changes to microbial biomass between treatments (Fig. 2g; Table S2; $P = 0.693$), showing that microbes respired intrinsically faster (that is, per unit of biomass) in the presence of lowland plants. These findings together imply that lowland plants accelerate alpine soil carbon loss by stimulating the activity of alpine soil microbes and the associated release of CO₂ through respiration.

We posit that lowland plants accelerated microbial activity by increasing the quantity of root exudates released into the soil, for four reasons. First, two-pool models of microbial respiration revealed that soils supporting any plant contained a soil carbon pool that was effectively absent from a bare soil control, and which was 1.5 ± 0.7 (mean ± SE) times larger under lowland plants than alpine plants (Fig. 2b; Table S2; $P = 0.046$). This carbon pool decayed at a similar rate between treatments (Fig.

120 2c; Table S2; $P = 0.631$), indicating that it differed in size, but likely not composition, between lowland
 121 and alpine treatments. Second, the same carbon pool was consumed rapidly by soil microbes (half-
 122 life = 5.1 ± 0.4 days), and we also found that lowland plant effects on microbial respiration were lost
 123 after nine days of incubation without plants (Fig. 2a). Plants thus supplied compounds to microbes
 124 that were depleted within this timeframe. A nine-day decay period corresponds to the time expected
 125 for newly photosynthesised CO_2 to be released through root exudation and respired by soil
 126 microbes^{21,22}, suggesting that this carbon pool was mostly root exudates. Third, lowland plant effects
 127 on the alpine soil system were rapid, occurring within a period of weeks in the glasshouse experiment
 128 and months in the field experiments. It is unlikely that lowland plants influenced microbial activity *via*
 129 litter inputs within this timeframe^{24,25}. Finally, dissolved organic matter contained a higher relative
 130 abundance of protein-like substances than humic-like and fulvic acid-like substances
 131 (Supplementary Fig. S5; Table S2; $P < 0.01$ in all cases) under lowland plants than alpine plants,
 132 also suggesting that lowland plants caused an enrichment in exudate-derived compounds²⁶.
 133 Together, these findings suggest that lowland plants induce soil carbon loss from alpine ecosystems
 134 by increasing root exudation relative to resident alpine plants, which accelerates soil microbial
 135 activity and the associated degradation and release of soil carbon as CO_2 ^{27,28}.

136

137 Plants adapted to different temperatures can possess distinct traits that have opposing effects on
 138 carbon cycle processes¹¹. We thus investigated whether lowland plants acted on the alpine soil
 139 system by introducing novel traits into the ecosystem. Publicly available trait data²⁹ relating to all
 140 species in the field experiments revealed that lowland plants on average possessed more productive
 141 traits than alpine plants (Supplementary Fig. S2a; $P = 0.025$). This was also the case in the
 142 glasshouse experiment, whereby trait measurements from all plants showed that lowland plants
 143 displayed more productive traits overall (Supplementary Fig. S2b; $P = 0.001$). Coupled to this, while
 144 lowland plants were planted at low abundance in the field experiments (mean $\pm$ SE relative cover:
 145 $4.7\% \pm 0.7\%$), the magnitude of soil carbon loss caused by lowland plant presence was greater in
 146 plots where lowland plants were more abundant overall (Supplementary Fig. S2e; $F_{1,14} = 5.79$, $P =$
 147 0.030). By contrast, we found no evidence that soil carbon loss in the field was alternatively related

to individual lowland species identity (Supplementary Fig. S2c,d) or the composition of the recipient alpine plant community (Supplementary Fig. S2,g).

Taken together, these findings confirm that lowland plants are functionally distinct from alpine plants. As a result, our results suggest that their establishment leads to a shift in plant community functioning that alters carbon cycle processes, with effects that intensify as lowland plants become more abundant in the community^{24,25}. However, it is also possible that lowland plants acted on the alpine soil system simply by introducing more biomass into the ecosystem. We investigated this possibility by summarising traits from the glasshouse experiment at the pot scale using a principal components analysis and testing for relationships between resulting axes of trait variation and alpine soil microbial respiration (Methods). We found that descriptors of plant biomass represented the most important axis of trait variation (Supplementary Fig. 4c) and were positively related to microbial respiration (Supplementary Fig. 4a; PC1: LR = 4.43, N = 20, $P = 0.035$). However, this relationship did not differ between lowland and alpine treatments (treatment $\times$ PC1: LR = 0.28, N = 20, $P = 0.596$), ruling it out as the mechanism through which lowland plants uniquely alter the alpine soil system. Instead, we found that trait variation represented by the second principal axis (for example, specific leaf area; Supplementary Fig. 4d) had opposing effects on microbial respiration between lowland and alpine treatments (Supplementary Fig. 4b; treatment $\times$ PC2: LR = 5.98, N = 20, $P = 0.015$). Lowland plants thus displayed relationships with the alpine soil system that were qualitatively distinct from resident alpine plants. While focussed work is now needed to pinpoint the specific physiological mechanism behind these differences, the existence of such a distinction reveals that the arrival of lowland plants in alpine ecosystems has the capacity to initiate a step-change in plant community functioning, with potential consequences for carbon cycle processes^{24,25}.

Lowland plant-induced carbon loss is pulsed

Soil microbial processes are constrained by multiple feedbacks within the soil system, such as substrate availability^{30,31} and microbial acclimation^{32–34}, that over time can slow, and eventually arrest, amplified soil carbon loss^{31,33,34}. We thus interrogated the state of the soil system in the field experiments after one season to explore the possible duration of lowland plant effects on alpine soil carbon loss. We focussed particularly on intrinsic rates (that is, per unit of biomass) of microbial respiration and growth as processes that promote soil carbon loss and gain, respectively^{28,35}, as well as microbial biomass as the scalar through which these processes affect soil carbon^{30,31}. We found that biomass-specific rates of microbial respiration in the field increased under warming in the presence, but not absence, of lowland plants (Fig. 2m; Table S1; $P = 0.038$). By contrast, biomass-specific rates of microbial growth were slower under warming but remained unaffected by lowland plant presence (Fig. 2n; Table S1; $P = 0.016$). These findings support observations from the glasshouse experiment that lowland plants accelerate intrinsic rates of microbial respiration (Fig. 2a,g), while also confirming that this persists for at least one season of field exposure. Moreover, by stimulating respiration, but not growth, lowland plants increased the capacity for alpine soils to lose carbon, but not to gain carbon *via* accumulation into microbial biomass^{28,35}.

Faster microbial respiration can only yield a loss of soil carbon if it occurs on a per unit of soil basis^{31,34,36}, which was the case in the glasshouse experiment (Fig. 2g). However, in the field we found that lowland plants caused a reduction of microbial biomass carbon in warmed plots (Fig. 2k; Table S1; $P < 0.001$). As such, although microbes always respired intrinsically faster in the presence of lowland plants, in the field they had become less abundant and so we observed no change to microbial respiration when expressed per unit of soil (Fig. 2i; Table S1; $P = 0.550$). Depressed microbial respiration per unit of soil was also evident at the ecosystem scale, in that warming accelerated total ecosystem respiration (that is, plant plus soil respiration) but its effect was dampened in plots containing lowland plants (Fig. 2h; Table S1; $P < 0.001$). The decline in microbial biomass likely took place because soil microbes are chiefly carbon limited, making their abundance tightly coupled to soil carbon content (here: $r_{26} = 0.61$, $P = 0.001$)³¹. Moreover, since intrinsic rates

of microbial growth were unaffected by lowland plants, a reduction in microbial biomass translated into a decline in microbial growth per unit of soil (Fig. 2j; Table S1; $P = 0.009$) – preventing any recovery of microbial biomass *via* growth³⁵. We thus suggest that as soil carbon was lost, microbial biomass also declined, and at the time of measurement this had entirely eliminated the influence of accelerated biomass-specific microbial respiration over the soil system. In short, the soil carbon loss observed in warmed field plots containing lowland plants must have both started and stopped within one season.

Our findings collectively suggest that the establishment of lowland plants in warming alpine ecosystems accelerates intrinsic rates of microbial respiration (Fig. 2a), which leads to a pulse of soil carbon release at baseline levels of microbial biomass (Fig. 1, Fig. 2g). However, resulting soil carbon loss leads to a decline in microbial biomass (Fig. 2k), a decrease in microbial respiration per unit of soil (Fig. 2i) and a cessation of further carbon loss from the ecosystem within a one-year timeframe (Fig. 2o)³¹. Here, we simulated a single event of lowland plant establishment, and at relatively low abundance in the community (mean $\pm$ SE relative cover: $4.7\% \pm 0.7\%$). Future work should thus address whether increases in lowland plant cover or repeated establishment events could induce additional pulses of alpine soil carbon loss. It is important to note that this interpretation does not consider the possibility that soil microbes acclimate to the presence of lowland plants, which could also yield a deceleration in microbial processes over time^{32–34}. Nevertheless, it remains true that biomass-specific microbial growth and respiration affect the soil system as a function of their biomass, and here changes to microbial biomass were sufficient to explain the behaviour of the soil system in both glasshouse and field experiments. We also found no evidence that soil microbes instead changed their resource use strategy in the presence versus absence of lowland plants (that is, carbon use efficiency; Fig. 2l; Table S1; $P < 0.001$; Tukey: $P = 0.996$), pointing away from microbial acclimation as an alternative mechanism.

Conclusions

In summary, we show that uphill migrations of native lowland plants can rapidly amplify the direct effects of climate warming on soil carbon loss from alpine ecosystems. This is important because it

243 demonstrates that plant range expansions between herbaceous plant communities have the
 244 potential to yield a positive feedback to climate warming, at least in the short term. These results
 245 contrast with expectations from woody species expansions in the Arctic, where shrubs with
 246 recalcitrant litter can slow carbon turnover in herbaceous communities and potentially yield a
 247 negative climate feedback^{14,15}. We thus suggest that the influence of range expanding species on
 248 ecosystem carbon dynamics depends on the relative trait composition of migrating species versus
 249 the recipient community³⁷, as well as subsequent effects on environmental processes¹⁴. While our
 250 findings demonstrate that lowland plants affect the rate of soil carbon loss in the short term, short-
 251 term experiments, such as ours, cannot resolve whether lowland plants will also affect the total
 252 amount of soil carbon lost in the long term. However, here soil carbon loss occurred despite a low
 253 abundance of lowland plants, and increased with total lowland plant cover. Given that both warming
 254 and associated plant migrations will intensify over time^{8,9}, our estimates of lowland plant impacts
 255 may be conservative – particularly if increases in lowland plant cover or repeated establishment
 256 events cause recurrent pulses of soil carbon loss. Moreover, while alpine ecosystems store only a
 257 small fraction of total global soil carbon^{1,2}, redistributions among herbaceous plant communities are
 258 occurring globally^{4–9}, including in similarly cool ecosystems in the Arctic that contain vast soil carbon
 259 stocks³⁸. This raises the potential for resulting climate feedbacks to also be widespread. Future work
 260 should focus on testing the conditions under which this feedback could occur in other ecosystems
 261 experiencing influxes of range expanding plant species, on quantifying how deeply it occurs in
 262 shallow alpine soils, and on estimating the magnitude of the climate feedback given both ongoing
 263 warming and variation in rates of species range shifts.

Materials & Methods

Field site description, experimental design. We made use of two independent alpine grassland transplant experiments that were established in Switzerland in the western Alps (46°12'40"N, 7°03'13"E)³⁹ and central Alps (46°52'20"N, 9°28'58"E) in autumn 2016. The experiments examine effects of climate warming on alpine ecosystems, including the arrival of lowland plants due to warming-induced uphill plant migrations (Supplementary Fig. S1). In the western Alps experiment the plant community is dominated by *Sesleria caerulea*, *Carex sempervirens* and *Vaccinium vitis-idea*. In the central Alps experiment the dominant species are *Nardus stricta*, *Alchemilla xanthochlora*, *Trifolium pratense* and *Anthyllis vulneraria*. Broadly, the experiments share the same design. Turfs (area = 1 m², depth = 20 cm) of intact alpine vegetation plus rhizosphere soil were removed from a high elevation control site (western Alps: 2200 m, MAT = 0.7 °C, TAP = 1448 mm; central Alps: 2000 m, MAT = 1.6 °C, TAP = 1151 mm; ref. ⁴⁰) and replanted either at a low elevation site to simulate climate warming (western Alps: 1400 m, MAT = 6.0 °C, TAP = 1236 mm, N = 10; central Alps: 1400 m, MAT = 4.4 °C, TAP = 1010 mm, N = 20; ref. ⁴⁰) or back into a high elevation site as a transplantation control (see below). At the low elevation site, we created an additional treatment to simulate the arrival of lowland plants under warming by planting a low abundance of lowland plant species in a grid design (13 cm between individuals), creating a lowland plant density of approximately 56 individuals per m² (4.7% ± 0.7% total cover) in a background alpine plant community. Lowland plants were positioned randomly in the grid, but with the same number of individuals per species per plot, and we controlled for planting disturbance in all remaining plots using alpine plants in place of lowland plants (see below). In the western Alps region, lowland plant species were *Achillea millefolium*, *Bellis perennis*, *Bromus erectus*, *Dactylis glomerata*, *Medicago lupulina*, *Plantago media* and *Salvia pratensis*, and individuals were planted into one side of each plot in a split-plot design (n = 10). In the central Alps experiment, lowland plant species were *Brachypodium pinnatum*, *Carex flacca*, *Carum carvi*, *Dactylis glomerata*, *Hypericum perforatum*, *Plantago lanceolata*, *Primula veris*, *Ranunculus bulbosus*, *Salvia pratensis*, *Silene vulgaris*, *Trifolium montanum* and *Viola hirta*, and individuals were planted into half of the transplanted turfs (n = 10). In the western Alps experiment, lowland individuals were pre-grown in a greenhouse in steam-sterilized field soil and planted into the plots in May 2017. In the central Alps experiment, lowland

individuals were taken from the surrounding vegetation and planted into the plots in autumn 2016. In both regions, soil was partially removed from lowland individuals prior to planting, and we performed an additional experiment to eliminate the possibility that small contaminations of lowland soil biota during planting could have modified alpine soil microbial community composition (see *Field Experiment Soil Biota Validation*, below). Overall, the experimental design in both regions yielded three treatments ($n = 10$ for each) arranged in a block design: warming only (W); warming plus low elevation plants (WL); and a high elevation control (C). One turf at the low elevation site in the western Alps experiment failed, leaving a total sample size of $N = 28$ and $N = 30$ in the western Alps and central Alps, respectively.

Field experiment controls and validation. We controlled for transplantation disturbance in both experiments by moving alpine control turfs back into a high elevation control site. In the western Alps experiment, alpine turfs were planted into different positions at their origin high elevation site. In the central Alps experiment, alpine turfs were planted into a new high elevation site at the same elevation. As such, we eliminated the possibility that alpine turfs changed simply because they were disturbed or were moved to a different location (that is, irrespective of elevation). The transplantation process had no effect on alpine soil carbon content, in that we found no difference in soil carbon content between replanted alpine control turfs at high elevation sites and undisturbed alpine soil in the surrounding area ($LR = 2.27$, $N = 16$, $P = 0.132$). We also controlled for potential effects of planting disturbance in all turfs not planted with lowland plants (that is, remaining turfs at low elevation and all control turfs at high elevation). In the western Alps experiment, we planted alpine species from the high elevation control site into the other side of alpine turfs at the low elevation site, as well as into all control turfs at the high elevation site. This was done in autumn 2016 using the same approach and randomized hexagonal grid design. In the central Alps experiment, we mimicked planting disturbance by digging up and replanting existing individuals in alpine turfs at the low elevation site not containing lowland plants, as well as all control turfs at the high elevation site. This was done in spring 2017 using the same grid design. It is important to note that we did not perform a reverse transplantation (that is, from low to high elevation), so we cannot entirely rule out the possibility that transplantation of any community to any new environment could yield a loss of soil

carbon. Nevertheless, it is well established that climate warming promotes soil carbon loss^{27,41,42}, and we found that soil from low elevation sites (that is, the potential end-member for warming alpine ecosystems) contained less carbon than soil from the alpine sites (70.1 ± 3.05 mg C g⁻¹ soil dry mass compared to 145.0 ± 5.29 mg C g⁻¹ soil dry mass; LR = 37.2, N = 27, $P < 0.001$), validating our expectation that alpine soil would lose carbon under warming. Elevation-based transplant experiments are a powerful approach for assessing warming effects on ecosystems^{11,43}, especially when they include rigorous disturbance controls (see above) and are performed in multiple locations where the common change from high to low elevation is temperature – here warming of between 2.8 °C (central alps) and 5.3 °C (western alps). While small variations in experimental design between regions, such in the order of planting in W and WL treatments, likely introduced some error when comparing experiments, we used both regions together so that we could identify treatment effects that were consistent among them and were thus not artefacts of experimental design.

Field experiment soil biota validation. We tested for the possibility that soil carbon loss was alternatively caused by low elevation soil microbes hitchhiking on lowland plants during planting. Specifically, we performed an experiment at the central Alps site to determine whether inoculating alpine plots with low elevation soil biota affected soil microbial community composition. We first generated a low elevation soil biota inoculum from rhizosphere soil from the same area where lowland plants were sourced and gently mixing and sieving it with tap water (following refs^{44,45}). We then added 3 L of inoculum to one half of each of 10 x 1 m² alpine turfs equivalent to those in the main experiment, followed by 3 L of water to rinse the inoculum from the vegetation and facilitate its infiltration into the soil. The other half of the plot (separated by an impermeable rubber barrier buried 15 cm deep into the soil around the perimeter of each subplot) received 6 L of water as a control. Soil inoculation was performed in mid-May and repeated in mid-June 2017. In October 2017, we sampled soils by pooling three soil cores ($\varnothing = 1$ cm, $d = 5$ cm) from each subplot, following which we determined soil microbial community composition by sequencing the 16S rRNA gene for bacteria/archaea and the ITS1 region for fungi⁴⁶. DNA was extracted (NucleoSpin Soil kit, Macherey-Nagel, Düren, Germany) and amplified using quadruplicate PCRs, alongside extraction and PCR blank controls. PCR products were purified (MinEluteTM PCR purification kit, Qiagen, Hilden,

Germany) prior to sequencing on a MiSeq sequencing platform (Illumina, San Diego, USA). Sequence data were processed using the OBITools software package⁴⁷ and following⁴⁸. Paired-end reads were merged, assigned to their respective samples and dereplicated, and sequences with too short read lengths and with only one read were excluded. Remaining sequences were clustered using the SUMACLUSt⁴⁹ algorithm and summarized into operational taxonomic units (OTUs) using the Infomap Community Detection Algorithm⁵⁰, following which we calculated per-sample relative OTU abundances for bacteria/archaea and fungi separately. We found that the alpine soil microbial community was resistant to the introduction of low elevation soil biota, in that inoculation had no effect on soil bacterial (Fig. S6a; PERMANOVA; $F_{1,9} = 0.75$, $P = 0.573$) or fungal (Fig. S6b; $F_{1,9} = 0.75$, $P = 0.774$) community composition. The inoculation represented a strong soil biota treatment relative to contamination by hitchhiking microbes. As such, even if some contamination by low elevation soil biota did occur, it was unlikely to have resulted in a change to the extant soil community, ruling out the possibility that contamination was alternatively responsible for the soil carbon loss observed when lowland plants were present in warmed alpine plots.

Field plant community composition. We surveyed the composition of alpine communities in summer 2017. This was done by dividing each plot into 5×5 cm cells and visually estimating the canopy cover of all species rooted in every grid cell. Species cover was approximated with an ordinal scale in which the first three cover categories correspond to 1/16, 1/8, and 1/4 of the grid cell area (25 cm^2), and subsequent cover categories were constant increases of 1/4 of the grid cell area. Plants were identified to species level whenever possible, although some taxa were identified only to genus or subfamily levels when species identity was unclear based on vegetative traits. Species cover (cm^2) was then calculated as the species-wise sum of cover estimates across all grid cells in the quarter of the plot used for soil and ecosystem respiration measurements (see below).

Field soil carbon content, microbial physiology, microbial biomass. We collected soil samples from all treatments in both field experiments ($N = 58$) in August 2017. In each plot, we created a composite sample from three cores ($\varnothing = 1 \text{ cm}$, approx. $d = 7 \text{ cm}$) no closer than 7 cm from a planted individual and from the same quarter of the plot used for ecosystem respiration measurements (see

below; Supplementary Fig. S1). We focused on rhizosphere soil for two reasons. First, alpine soils are shallow and alpine plants are shallow-rooting, making the rhizosphere the most logical target for a first assessment of warming and lowland plant effects on alpine soil carbon cycling. Second, we wanted to avoid any potential contamination from the base of the turf (depth = 20 cm). Soils were kept cool, transported immediately to the laboratory, sieved (2 mm mesh size), adjusted to 60% of water holding capacity (WHC) and pre-incubated for three days at field temperature prior to measurements. We measured microbial biomass carbon (mg per g dry mass) *via* chloroform fumigation extraction followed by analysis on a TOC-VCPH/CPNTNM-1 analyzer (Shimadzu; 48h incubation period; 1 M KCl extraction for fumigated and non-fumigated samples; conversion factor 0.45)⁵¹. While this approach also yielded measurements of dissolved organic carbon (DOC; mg C per g dry mass), treatment effects on this variable echoed those of microbial biomass carbon (Table S1) so we did not interpret them separately. Soil organic carbon content (%) was measured on dry soil (60 °C for 48h) with a Carlo Erba 1110 elemental analyzer (EA, CE Instruments) coupled to a Delta Plus IRMS via a ConFlo III (Thermo Fisher). We additionally quantified microbial growth, respiration and carbon use efficiency in soils from the western Alps experiment (N = 28) using the incorporation of ¹⁸O into DNA³¹. Briefly, we incubated 500 mg of soil for 24h at field temperature following addition of either ¹⁸O-H₂O to 20 atom% enrichment and 80% of WHC or the same volume of molecular grade non-labelled H₂O as a natural abundance control. Gas samples were taken at the start and end of the 24h incubation and analyzed for CO₂ concentration with a Trace GC Ultra (Thermo Fisher) to determine microbial respiration (μg C per g dry mass per h). At the end of the incubation, soil was snap-frozen in liquid nitrogen, and DNA was extracted (FastDNA SPIN Kit for Soil, MP Biomedical), quantified (Quant-iT PicoGreen dsDNA Assay Kit, Thermo Fisher) and analyzed for ¹⁸O abundance and total oxygen content using a thermochemical EA coupled to a Delta V Advantage IRMS via a ConFlo III (Thermo Fisher). Following ref. ³¹, we calculated total DNA production during the 24h incubation (μg DNA per gram dry mass) and coupled it with measures of microbial biomass carbon content to derive microbial growth (μg C per gram dry mass per h) and carbon use efficiency (%).

408 **Field ecosystem respiration.** We measured rates of ecosystem respiration (g CO₂ per m² per h)
 409 on multiple dates (western Alps: 7 dates; central Alps: 3 dates) between July and August 2017. One
 410 month prior to the first measurement, we installed permanent gas sampling collars ($h = 10$ cm, $\varnothing =$
 411 30 cm) in each plot to a depth of 5 cm to create an airtight seal at the soil surface¹¹. Measurements
 412 were made by enclosing plants and soil within the collars with an opaque chamber ($h = 50$ cm, $\varnothing =$
 413 30 cm) and, following a 30s equilibration period, monitoring the increase in CO₂ concentration at 5s
 414 intervals over a 60s period (Vaisala GMP343 CO₂ probe attached to an HM70 meter, Vaisala).
 415 Chambers contained a fan to mix the headspace during measurements and a small sealable hole
 416 on the top that was opened when placing the chamber on sampling collars to avoid pressurization
 417 inside the chamber. Soil temperature and moisture were recorded for each measurement and used
 418 alongside measurements of air temperature, air pressure, enclosure volume and soil surface area
 419 to calculate the CO₂ flux¹¹. Where necessary, we repeated measurements in the field to attain a
 420 visually consistent CO₂ change over time, and during data processing we discarded fluxes with an
 421 r -squared of less than 0.9 for this relationship.

422
 423 **Glasshouse experiment design.** We established a six-week pot experiment to test for effects of
 424 lowland plants on the alpine soil system. Specifically, we filled pots ($\varnothing = 10$ cm, depth = 9 cm) with
 425 sieved soil (mesh size 4 mm) from the high elevation site of the western Alps field experiment, and
 426 planted them with lowland species (*Bromus erectus*, *Salvia pratensis*, *Medicago lupulina*) or alpine
 427 species (*Phleum alpinum*, *Anthyllis vulneraria* ssp. *alpestris*, *Plantago alpina*) to create a lowland
 428 only treatment, an alpine only treatment and a bare soil control (N = 30). We selected a grass, herb
 429 and legume species for each treatment, all of which were found naturally at the low (lowland
 430 treatment) or high (alpine treatment) elevation sites in the western Alps field experiment. Vegetated
 431 treatments contained two individuals of each species planted in a circular pattern such they were
 432 never adjacent to conspecifics or plants of the same functional type (i.e. grass, herb, legume). The
 433 original design also included a mixed community treatment containing one individual of each lowland
 434 and alpine species. However, the mixed treatment had double the species richness of other
 435 vegetated treatments and contained an equal cover of lowland and alpine plants (compared to < 5%
 436 lowland plant cover in field plots), so we focused our analyses on the comparison between alpine

and lowland plant treatments to understand how lowland plants affect the soil differently from alpine plants. All plants were pre-grown from seed provided by commercial suppliers (www.hauenstein.ch, www.schutzfilisur.ch, www.saatzbau.at) in steam-sterilized potting soil for two to three weeks to approximately the same biomass and root-washed immediately prior to planting. Pots were arranged in a randomized block design ($n = 10$) in a greenhouse under standardized growing conditions (22 °C; 50% humidity; no artificial light), and were watered every two to three days as needed. After six weeks, we destructively harvested the plants and soil to characterize treatment effects on plant physiology and the soil system.

Glasshouse plant physiology. We measured maximum leaf-level photosynthesis (photosynthetic capacity; $A_{\max}$; $\mu\text{mol CO}_2$ per m^2 per s) and stomatal conductance (g_s ; $\mu\text{mol H}_2\text{O}$ per m^2 per s) under optimum conditions on one individual per species per pot using an open path infrared gas analyzer connected to a 2.5 cm chamber (CIRAS-2 and PLC-6, PP Systems)¹¹. Readings were taken once for each individual and corrected using measurements of leaf area (cm^2) on the same leaf, which we also dried and weighed to determine specific leaf area (SLA; cm^2 per gram). Following soil pore water sampling (below), we quantified total, aboveground and belowground biomass of all individuals in the experiment by destructively sampling, washing, drying (60 °C for 48h) and weighing all plant tissue.

Glasshouse soil pore water analysis. We collected soil pore water from all pots prior to soil sampling (see below) to determine treatment effects on dissolved organic matter (DOM) quality. Soil water was sampled by leaching pots twice with 50 mL sterile tap water, combining and filtering leached water (filter pore size 0.2 μm) and measuring absorbance (200-900 nm with a 1nm increment; 1 cm quartz cuvette; UV-vis spectrophotometer, Shimadzu) and fluorescence intensity (excitation: 240-450 nm with a 5 nm increment; emission: 290-550 nm with a 2.5 nm increment; Fluorolog-3 spectrometer, Horiba). We corrected absorbance spectra against a milli-Q water blank, calculated Napierian absorption coefficients and used these data to derive absorbance at 350 nm (a_{350} ; a proxy for DOM content). We expressed fluorescence data as total fluorescence (F_{tot} ; Raman Units) and the fluorescence index (FI), i.e. the ratio of emission intensity at 470 nm versus 520 nm

at an excitation wavelength of 370 nm (a proxy for microbially-derived DOM⁵²). We also derived excitation-emission matrices (EEMs) to quantify the relative abundance of different components of DOM through PARAFAC algorithms⁵³. Following preprocessing (i.e. Raman scattering removal and standardization to Raman Units) and normalization⁵⁴, we used the drEEM toolbox⁵⁵ in MATLAB (Mathworks) to obtain a six-component PARAFAC model. The model was validated with split-half analysis, random initialization and visualization of EEM residuals, after which we calculated the relative abundance of each component as the maximum fluorescence signal of the component against the total maximum fluorescence signal of all components. Finally, we classified components against classical EEM nomenclature (Table S4; refs ^{56,57}). While PARAFAC modelling provides a means to discriminate between DOM sources (e.g. components C3 and C4; Table S4), we did not physically separate plant- and microbe-derived DOM when sampling soil pore water. As such, observed changes to DOM composition were likely a result of changes to both plant and microbial processes (see Main Text).

Glasshouse soil carbon pools, microbial respiration. We took soil cores ($\varnothing = 1$ cm, $d = 7$ cm) from the center of each pot (i.e. equidistant from all individuals) to quantify soil carbon pools and microbial respiration. Soils were sieved (2 mm mesh size), adjusted to 60% of their WHC and pre-incubated at 22 °C for three days prior to measurements. We quantified concentrations of microbial biomass carbon in all soils as for field soil (see above; mg C per g dry mass). We determined microbial respiration by incubating 8 g sieved soil in the dark at 22 °C for a further six-week period without plants. We measured microbial respiration daily for the first four days, then approximately biweekly until the end of the six-week period. We measured CO₂ concentrations (EGM-4, PP Systems) of gas samples taken approximately 24h after flushing enclosed vials ($v = 126$ mL) with CO₂-free air, and calculated microbial respiration (μ g C per g dry mass per h) using accompanying measurements of enclosure volume, air pressure and soil mass¹¹. After each measurement, soils were weighed and, when necessary, re-adjusted to 60% of their WHC.

Two-pool mixed models of soil carbon mineralization. We described the kinetics of soil carbon mineralization during the follow-up incubation of glasshouse soil by employing a two-pool mixed

model to microbial respiration data⁵⁸. Specifically, we constructed a model that considered a first pool that followed first-order decomposition kinetics (i.e. a small soil carbon pool with a fast turnover) and a second pool that followed zero-order kinetics (i.e. decay rate was constant over the incubation period, representing a large soil carbon pool with a slow turnover):

$$C_{min} = C_l(1 - e^{-t k_l}) + B \cdot t$$

where C_{min} is the cumulative CO₂ respired (μg C g⁻¹) over the incubation time t (d), C_l and k_l are the size (μg C g⁻¹) and decomposition constant (d⁻¹) of the first-order kinetics carbon pool, respectively, and B is the basal respiration rate (μg C g⁻¹ d⁻¹) of the zero-order kinetics carbon pool. Given that C_{min} and t were known, we ran the two-pool mixed model with the `nlsList` function (`maxiter` = 100, `tol` = 1×10^{-5} , `minFactor` = 1/1024) in R⁵⁹ using the package “nlme”⁶⁰ for each pot separately to estimate values for C_l , k_l and B . Models failed to converge for one sample, and we excluded four additional samples for which models made negative pool estimates (N = 25).

Statistical analysis. The majority of statistical test outputs appear as P -values in the Main Text, with full outputs being provided in Tables S1 to S3. See text below for exceptions. Data analyses and visualization were undertaken in R⁵⁹ using the packages “cowplot”⁶¹, “drake”⁶², “emmeans”⁶³, “nlme”⁶⁰, “tidyverse”⁶⁴ and “vegan”⁶⁵. Where necessary, region was accounted for in statistical models as a fixed effect, since two levels was too few to be included as a random intercept term. As such, region effects are presented (Tables S1, S3), but not interpreted. Statistical methods are described as they appear in the Main Text.

Warming and warming plus lowland plant effects on soil carbon content: we used field data to test for treatment (C, W, WL) effects on soil carbon content using an LME model including region as a fixed effect and block as a random intercept term (see Table S1 for test output). We also calculated how much more soil carbon loss occurred in the presence versus absence of lowland plants by fitting a single LME testing for effects of soil temperature (plot means of all sampling dates), the presence or absence of lowland plants and their interaction on soil carbon content (see Supplementary Fig.

524 S2 for test outputs). In order to compare slopes between C-W and C-WL treatment combinations,
525 we duplicated C treatment values, assigned one set to each C-W and C-WL pair and accounted for
526 this using a random intercept term. We also included region as a random slope term. We then tested
527 how much more soil carbon loss would occur per °C of warming when lowland plants are present by
528 calculating the relative difference ($\% \pm 95\%$ confidence intervals) between the C-WL and C-W
529 slopes.

530

531 Plant mechanisms for soil carbon loss: we expressed soil carbon content as the block-wise percent
532 changes between WL and W treatments to derive a metric for lowland plant induced soil carbon loss
533 (i.e. % soil carbon remaining). We tested for lowland species identity effects on soil carbon remaining
534 using separate LMEs for each region (due to different lowland species pools) that tested for individual
535 species cover (cm^2) effects on soil carbon remaining in WL plots, including species as a random
536 intercept term (see Supplementary Fig. S2 for test outputs). We also performed separate linear
537 models including species as fixed effects, none of which were statistically significant (Table S3). We
538 tested for effects of total lowland species cover (cm^2) on soil carbon remaining in WL plots using a
539 linear model also including region as a fixed effect. We summarized alpine community composition
540 in W and WL treatments using an NMDS (Hellinger distance matrix, two axes), following which we
541 expressed NMDS scores as the block-wise percent changes between W and WL treatments to
542 derive metrics for lowland plant induced alpine plant community change, and used a linear model
543 that tested for effects of changes to NMDS scores on soil carbon remaining in WL plots including
544 region as a fixed effect (see Supplementary Fig. S3 for test outputs). For reference, raw NMDS
545 scores did not significantly differ between W and WL treatments (LME: NMDS #1: LR = 1.57, P =
546 0.210; NMDS #2: LR = 2.67, P = 0.102). Finally, we tested for differences between alpine and
547 lowland species traits in the field experiment using a PERMANOVA on a PCA of species mean trait
548 values ($\log_{10}$ plant height, $\log_{10}$ leaf area, $\log_{10}$ seed mass, SLA, leaf carbon, leaf nitrogen) from the
549 TRY database²⁹ for all species present (where necessary, missing values were gap-filled
550 following⁶⁶), and in the glasshouse experiment using a PERMANOVA on a PCA of physiological
551 measurements (total biomass, leaf biomass, root biomass, SLA, $A_{\max}$, g_s) for all plants (see
552 Supplementary Fig. S3 for test outputs). Traits were selected to explore broad life history differences

between alpine and lowland plant species⁶⁷, but were not used to identify the specific trait(s) responsible for lowland plant effects on alpine soil carbon loss. We used TRY traits for field species due to the large number of species present in the communities (N = 118), but used measured traits in the glasshouse experiment to provide direct evidence of physiological differences between alpine and lowland species.

Lowland versus alpine plant effects on the soil system: we used the glasshouse experiment to test for lowland versus alpine plant effects on soil microbial biomass carbon, the size (C_i) and decay rate (k_i) of the first modelled soil carbon pool and all variables relating to soil DOM quality using LMEs including block as a random intercept term. We tested for effects of treatment, time and their interaction on soil microbial respiration using the same LME structure but including the bare soil treatment. In all cases, including/excluding the bare soil treatment had no effect over model outcomes, but mean $\pm$ SE values for this treatment are included in figures for reference. Finally, we explored how plant physiology in lowland and alpine treatments affected initial rates of soil microbial respiration. We defined initial microbial respiration as the pot-wise mean respiration rates of the first nine days of soil incubation (that is, while treatment effects persisted; see Fig. 2a), and expressed pot-level plant physiology using scores from the first two axes of a principal components analysis performed on sums of total, root and shoot biomass and biomass-weighted means of SLA, A_{max} and g_s . We then tested for effects of treatment, PC1 and PC2 scores and their interaction on initial microbial respiration using an LME including block as a random intercept term. Statistical test outputs for LMEs are provided in Table S2, except for treatment and plant physiology effects on microbial respiration, which appear in the Main Text.

Sequence of warming and lowland plant effects on alpine soil carbon loss: we used field data to test for treatment (C, W, WL) effects on soil microbial biomass carbon and soil DOC using LME models including region as a fixed effect and block as a random intercept term. We tested for treatment effects on ecosystem respiration using an LME that included region as a fixed effect and sampling date nested in block as a random intercept term. We tested for treatment effects on soil microbial growth and respiration (per unit of soil and per unit of microbial biomass) and carbon use efficiency

in the western Alps experiment using LMEs including block as a random intercept term. Statistical test outputs for these variables are provided in Table S1. We also used Pearson Product Moment correlations to test for associations between soil microbial biomass and soil carbon content, microbial respiration and growth in the western Alps experiment, irrespective of experimental treatment.

Acknowledgements

JMA and HN are funded by the European Research Council (grant no. 678841). JMA and TWNW are funded by the Swiss National Science Foundation (grant no. 31003A-176044). KG is funded by the Swiss National Science Foundation (grant no. PZ00P2-174047). SB and the Central Alps experiment are funded by the Swiss National Science Foundation (grant no. 31003A-173210).

Author contributions

TWNW, AB and JMA conceived the study. AR, AB and JMA provided resources for field and laboratory experiments. SB, TM and HN established and maintained field experiments. TWNNW, KG and TG undertook the field work. TWNNW, KG, TG, PM, CS, AB and JMA undertook the glasshouse experiment. TWNNW, KG, TG, PM, CS and AR did the laboratory work. TL performed the soil pore water sampling and associated analyses. SB and TM performed the soil inoculation experiment and associated analyses. TWNNW did the data analysis, with support from DR, TWC and JMA. TWNNW wrote the manuscript in close collaboration with JMA, and with support from all co-authors.

Competing interests

The authors declare no competing financial interests.

References

1. Streit, K. *et al.* Soil warming alters microbial substrate use in alpine soils. *Glob. Change Biol.* **20**, 1327–1338 (2014).
2. Parker, T. C., Subke, J.-A. & Wookey, P. A. Rapid carbon turnover beneath shrub and tree vegetation is associated

- 611 with low soil carbon stocks at a subarctic treeline. *Glob. Change Biol.* **21**, 2070–2081 (2015).
- 612 3. Knowles, J. F., Blanken, P. D., Lawrence, C. R. & Williams, M. W. Evidence for non-steady-state carbon emissions
613 from snow-scoured alpine tundra. *Nat. Commun.* **10**, 1306 (2019).
- 614 4. Parmesan, C. Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**,
615 637–669 (2006).
- 616 5. Lenoir, J. & Svenning, J. C. Climate-related range shifts - a global multidimensional synthesis and new research
617 directions. *Ecography* **38**, 15–28 (2015).
- 618 6. Pecl, G. T. *et al.* Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being.
619 *Science* **355**, (2017).
- 620 7. Dolezal, J. *et al.* Vegetation dynamics at the upper elevational limit of vascular plants in Himalaya. *Sci. Rep.* **6**,
621 24881 (2016).
- 622 8. Rumpf, S. B. *et al.* Range dynamics of mountain plants decrease with elevation. *Proc. Natl. Acad. Sci. USA* **115**,
623 1848–1853 (2018).
- 624 9. Steinbauer, M. J. *et al.* Accelerated increase in plant species richness on mountain summits is linked to warming.
625 *Nature* **556**, 231–234 (2018).
- 626 10. Wallingford, P. D. *et al.* Adjusting the lens of invasion biology to focus on the impacts of climate-driven range shifts.
627 *Nat. Clim. Chang.* (2020). doi:10.1038/s41558-020-0768-2
- 628 11. Walker, T. W. N. *et al.* Plastic and genetic responses of a common sedge to warming have contrasting effects on
629 carbon cycle processes. *Ecol. Lett.* **22**, 159–169 (2019).
- 630 12. Liao, C. *et al.* Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytol.* **177**,
631 706–714 (2008).
- 632 13. Waller, L. P. *et al.* Biotic interactions drive ecosystem responses to exotic plant invaders. *Science* **368**, 967–972
633 (2020).
- 634 14. Pearson, R. G. *et al.* Shifts in Arctic vegetation and associated feedbacks under climate change. *Nat. Clim. Chang.*
635 **3**, 673–677 (2013).
- 636 15. Cornelissen, J. H. C. *et al.* Global negative vegetation feedback to climate warming responses of leaf litter
637 decomposition rates in cold biomes. *Ecol. Lett.* **10**, 619–627 (2007).
- 638 16. Körner, C. *Alpine Plant Life: Functional Ecology of High Mountain Ecosystems*. (Springer, 2003).
- 639 17. Pepin, N. *et al.* Elevation-dependent warming in mountain regions of the world. *Nat. Clim. Chang.* **5**, 424–430
640 (2015).
- 641 18. Lenoir, J., Gégout, J. C., Marquet, P. A., de Ruffray, P. & Brisse, H. A significant upward shift in plant species
642 optimum elevation during the 20th century. *Science* **320**, 1768–1771 (2008).
- 643 19. Hagedorn, F., Gavazov, K. & Alexander, J. M. Above- and belowground linkages shape responses of mountain
644 vegetation to climate change. *Science* **365**, 1119–1123 (2019).
- 645 20. Budge, K., Leifeld, J., Hiltbrunner, E. & Fuhrer, J. Alpine grassland soils contain large proportion of labile carbon

- 646 but indicate long turnover times. *Biogeosciences* **8**, 1911–1923 (2011).
- 647 21. Hafner, S. *et al.* Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture
648 revealed by $^{13}\text{CO}_2$ pulse labeling. *Glob. Change Biol.* **18**, 528–538 (2012).
- 649 22. Studer, M. S., Siegwolf, R. T. W. & Abiven, S. Carbon transfer, partitioning and residence time in the plant-soil
650 system: a comparison of two $^{13}\text{CO}_2$ labelling techniques. *Biogeosciences* **11**, 1637–1648 (2014).
- 651 23. Plante, A. F., Stone, M. M. & McGill, W. B. in *Soil microbiology, ecology and biochemistry* 245–272 (Elsevier,
652 2015). doi:10.1016/B978-0-12-415955-6.00009-8
- 653 24. De Deyn, G. B., Cornelissen, J. H. C. & Bardgett, R. D. Plant functional traits and soil carbon sequestration in
654 contrasting biomes. *Ecol. Lett.* **11**, 516–531 (2008).
- 655 25. Cornwell, W. K. *et al.* Plant species traits are the predominant control on litter decomposition rates within biomes
656 worldwide. *Ecol. Lett.* **11**, 1065–1071 (2008).
- 657 26. Jones, D. L., Nguyen, C. & Finlay, R. D. Carbon flow in the rhizosphere: carbon trading at the soil–root interface.
658 *Plant Soil* **321**, 5–33 (2009).
- 659 27. Melillo, J. M. *et al.* Soil warming and carbon-cycle feedbacks to the climate system. *Science* **298**, 2173–2176
660 (2002).
- 661 28. Allison, S. D., Wallenstein, M. D. & Bradford, M. A. Soil-carbon response to warming dependent on microbial
662 physiology. *Nat. Geosci.* **3**, 336–340 (2010).
- 663 29. Kattge, J. *et al.* TRY plant trait database - enhanced coverage and open access. *Glob. Change Biol.* **26**, 119–188
664 (2020).
- 665 30. Hartley, I. P., Hopkins, D. W., Garnett, M. H., Sommerkorn, M. & Wookey, P. A. Soil microbial respiration in arctic
666 soil does not acclimate to temperature. *Ecol. Lett.* **11**, 1092–1100 (2008).
- 667 31. Walker, T. W. N. *et al.* Microbial temperature sensitivity and biomass change explain soil carbon loss with warming.
668 *Nat. Clim. Chang.* **8**, 885–889 (2018).
- 669 32. Crowther, T. W. & Bradford, M. A. Thermal acclimation in widespread heterotrophic soil microbes. *Ecol. Lett.* **16**,
670 469–477 (2013).
- 671 33. Bradford, M. A. *et al.* Cross-biome patterns in soil microbial respiration predictable from evolutionary theory on
672 thermal adaptation. *Nat. Ecol. Evol.* **3**, 223–231 (2019).
- 673 34. Melillo, J. M. *et al.* Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming
674 world. *Science* **358**, 101–105 (2017).
- 675 35. Liang, C., Schimel, J. P. & Jastrow, J. D. The importance of anabolism in microbial control over soil carbon storage.
676 *Nat. Microbiol.* **2**, 17105 (2017).
- 677 36. Prommer, J. *et al.* Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at
678 higher plant diversity. *Glob. Change Biol.* **26**, 669–681 (2020).
- 679 37. Bjorkman, A. D. *et al.* Plant functional trait change across a warming tundra biome. *Nature* **562**, 57–62 (2018).
- 680 38. Tarnocai, C. *et al.* Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochem.*

681 *Cycles* **23**, (2009).

682 39. Nomoto, H. A. & Alexander, J. M. Drivers of local extinction risk in alpine plants under warming climate. *Ecol. Lett.*
683 **24**, 1157–1166 (2021).

684 40. Karger, D. N. *et al.* Climatologies at high resolution for the earth's land surface areas. *Sci. Data* **4**, 170122 (2017).

685 41. Crowther, T. W. *et al.* Quantifying global soil carbon losses in response to warming. *Nature* **540**, 104–108 (2016).

686 42. Davidson, E. A. & Janssens, I. A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate
687 change. *Nature* **440**, 165–173 (2006).

688 43. Alexander, J. M., Diez, J. M. & Levine, J. M. Novel competitors shape species' responses to climate change.
689 *Nature* **525**, 515–518 (2015).

690 44. van de Voorde, T. F. J., van der Putten, W. H. & Bezemer, T. M. Soil inoculation method determines the strength of
691 plant–soil interactions. *Soil Biol. Biochem.* **55**, 1–6 (2012).

692 45. De Vries, F. T., Bracht Jørgensen, H., Hedlund, K. & Bardgett, R. D. Disentangling plant and soil microbial controls
693 on carbon and nitrogen loss in grassland mesocosms. *J Ecol* **103**, 629–640 (2015).

694 46. Herbold, C. W. *et al.* A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of
695 diverse target genes. *Front. Microbiol.* **6**, 731 (2015).

696 47. Boyer, F. *et al.* obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**, 176–182
697 (2016).

698 48. Zinger, L. *et al.* Soil community assembly varies across body sizes in a tropical forest. *BioRxiv* (2017).
699 doi:10.1101/154278

700 49. Mercer, C., Boyer, F., Bonin, A. & Coissac, E. *SUMATRA and SUMACLUSt: fast and exact comparison and*
701 *clustering of sequences*. (LECA, 2013).

702 50. Rosvall, M. & Bergstrom, C. T. Maps of random walks on complex networks reveal community structure. *Proc. Natl.*
703 *Acad. Sci. USA* **105**, 1118–1123 (2008).

704 51. Vance, E. D., Brookes, P. C. & Jenkinson, D. S. An extraction method for measuring soil microbial biomass C. *Soil*
705 *Biol. Biochem.* **19**, 703–707 (1987).

706 52. McKnight, D. M. *et al.* Spectrofluorometric characterization of dissolved organic matter for indication of precursor
707 organic material and aromaticity. *Limnol. Oceanogr.* **46**, 38–48 (2001).

708 53. Stedmon, C. A., Markager, S. & Bro, R. Tracing dissolved organic matter in aquatic environments using a new
709 approach to fluorescence spectroscopy. *Mar Chem* **82**, 239–254 (2003).

710 54. Zepp, R. G., Sheldon, W. M. & Moran, M. A. Dissolved organic fluorophores in southeastern US coastal waters:
711 correction method for eliminating Rayleigh and Raman scattering peaks in excitation–emission matrices. *Mar Chem*
712 **89**, 15–36 (2004).

713 55. Murphy, K. R., Stedmon, C. A., Graeber, D. & Bro, R. Fluorescence spectroscopy and multi-way techniques.
714 PARAFAC. *Anal. Methods* **5**, 6557 (2013).

715 56. Fellman, J. B., Hood, E. & Spencer, R. G. M. Fluorescence spectroscopy opens new windows into dissolved

716 organic matter dynamics in freshwater ecosystems: A review. *Limnol. Oceanogr.* **55**, 2452–2462 (2010).

717 57. *Aquatic organic matter fluorescence*. (Cambridge University Press, 2014). doi:10.1017/CBO9781139045452

718 58. Bonde, T. A. & Rosswall, T. Seasonal variation of potentially mineralizable nitrogen in four cropping systems. *Soil*

719 *Sci. Soc. Am. J.* **51**, 1508–1514 (1987).

720 59. R Core Team. *R: A language and environment for statistical computing*. (R Foundation for Statistical Computing,

721 2014).

722 60. Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. *nlme: Linear and Nonlinear Mixed Effects Models*. (R Project,

723 2019).

724 61. Wilke, C. O. *cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2.”* (R Project, 2019).

725 62. Michael Landau, W. The drake R package: a pipeline toolkit for reproducibility and high-performance computing.

726 *JOSS* **3**, 550 (2018).

727 63. Lenth, R. *Estimated Marginal Means, aka Least-Squares Means*. (R Project, 2020).

728 64. Wickham, H. *et al.* Welcome to the tidyverse. *JOSS* **4**, 1686 (2019).

729 65. Oksanen, J. *et al.* *vegan: Community Ecology Package*. (R Project, 2019).

730 66. Schrod, F. *et al.* BHPMF - a hierarchical Bayesian approach to gap-filling and trait prediction for macroecology and

731 functional biogeography. *Glob. Ecol. Biogeogr.* **24**, 1510–1521 (2015).

732 67. Díaz, S. *et al.* The global spectrum of plant form and function. *Nature* **529**, 167–171 (2016).

733

Supplementary Figure Legends

Fig. S1 | Experimental design. (a) In both field experiments, intact alpine turfs containing alpine plant communities plus rhizosphere soil were removed from their high elevation home sites and either replanted at the home site (negative control; C) or transplanted to a low elevation recipient site to simulate climate warming (W, WL). At low elevation, half of the turfs were planted with a low abundance of lowland plant species to additionally simulate the arrival of migrating plant species in the ecosystem (WL). Both other treatments (i.e. C, W) were planted with the same abundance of alpine plant species as a disturbance control. (b) The layout of W and WL treatments at the low elevation site differed between western and central alps experiments. In the central alps, W and WL treatments were established in distinct turfs planted adjacently in a block design ($N = 10$). In the western alps, W and WL treatments were established in different halves of the same turf in a split-plot design ($N = 10$).

Fig. S2 | Alpine and lowland plant effects on soil carbon loss. (a,b) Trait separation of lowland (yellow) and alpine (green) plant species, displayed as PC1 and PC2 scores from PCAs containing (a) TRY data for species in field experiments ($N = 242$; $F_{1,240} = 3.98$, $P = 0.003$) or (b) measured data from a glasshouse experiment on selected species ($N = 109$; $F_{1,107} = 17.69$, $P = 0.001$). Arrows show loadings and P -values refer to alpine-lowland comparison (PERMANOVAs). (c-g) Relationships between soil carbon loss (% remaining) and cover (cm^2) of each lowland plant species in the (c) western Alps ($N = 36$; LME: $\text{LR} = 0.62$, $P = 0.4302$) and (d) central Alps ($N = 80$; LME: $\text{LR} = 0.33$, $P = 0.5666$) experiments, (e) total lowland plant cover (cm^2 ; $N = 19$; see Main Text) and (f,g) alpine plant community composition (% change in NMDS axes; $N = 18$; linear models: NMDS #1: $F_{1,14} = 1.19$, $P = 0.2934$, NMDS #2: $F_{1,14} = 3.17$, $P = 0.0966$; region: $F_{1,14} = 1.29$, $P = 0.2755$). For (c,d), non-significant species-wise fit lines (yellow) are also displayed.

Fig. S3 | Soil temperature and lowland plant effects on alpine soil carbon loss. Relationship between soil temperature ($^{\circ}\text{C}$) and soil carbon content (C ; mg g^{-1} dry mass) in alpine turfs exposed to warming (W; light grey), warming plus lowland plants (WL; dark grey) or an ambient control (C; white). Statistics describe effects of warming (W; $\text{LR} = 17.65$, $P < 0.0001$), lowland plants (L; $\text{LR} = 11.77$, $P = 0.0006$) and their interaction ($W \times L$; $\text{LR} = 11.72$, $P = 0.0006$) from one linear mixed effects model ($N = 78$), but are visualized with separate fit lines (including slopes) for C-W (dashed lines) and C-WL (solid lines) comparisons in the western Alps (triangles) and central Alps (circles) field experiments.

Fig. S4 | Alpine and lowland plant effects on alpine soil microbial respiration. (a,b) Relationships between plant physiology, expressed as (a) PC1 and (b) PC2 scores from PCAs of pot-level measurements, and rates of soil microbial respiration during the first nine days of incubation (initial R ; $\mu\text{g C g}^{-1} \text{ h}^{-1}$) in alpine (green) versus lowland (yellow) treatments of the glasshouse experiment. Fit lines describe relationships as determined by a single linear mixed effects model including treatment, PC1, PC2 and treatment $\times$ PC# interactions ($N = 20$; Main Text), with different colours in (b) indicating a significant treatment $\times$ PC2 interaction. (c,d) Loadings of plant physiological measurements on (c) PC1 and

(d) PC2 axes. Total, belowground and aboveground biomass are sums of all plants per pot and specific leaf area (SLA), maximum photosynthetic capacity (A_{max}) and stomatal conductance (g_s) are pot-wise community weighted mean (CWM) values.

Fig. S5 | Alpine and lowland plant effects on alpine soil dissolved organic matter. Effects of alpine (A) and lowland (L) plants on the relative abundance (%; means $\pm$ SEs) of six dissolved organic matter components (C1-C6) in soil pore water extracted from the glasshouse experiment. Grey ribbons are SEs for a bare soil control. Components and associated compound families were derived from PARAFAC modelling. Treatment effects ($P < 0.05$) are indicated by asterisks (LMEs; see Table S2 for model outputs).

Fig. S6 | Soil inoculation effects on alpine soil community composition. Effects of low elevation soil biota inoculation on (a) bacterial and (b) fungal community composition in alpine plots ($N = 10$ in both cases). Axes show the first two components of principal coordinates analyses (PCoA; Bray Curtis distance) performed on the relative abundances of bacterial and fungal operational taxonomic units, respectively. P -values are from PERMANOVAs testing for inoculation effects on the same distance matrices.

Supplementary Tables

Table S1. Linear mixed effects model outputs for effects of field treatment (control, warming, warming plus lowland plants), region and their interaction on soil variables.

Response	Treatment		Region		Treatment × Region	
	LR	<i>P</i>	LR	<i>P</i>	LR	<i>P</i>
Soil carbon content	23.16	< 0.0001	34.53	< 0.0001	4.64	0.0984
Ecosystem respiration	49.61	< 0.0001	13.08	0.0045	12.98	0.0015
Microbial biomass C	33.52	< 0.0001	13.64	0.0034	5.35	0.0688
Microbial growth (per gram soil)	9.33	0.0094	-	-	-	-
Microbial respiration (per gram soil)	1.20	0.5500	-	-	-	-
Microbial growth (biomass-specific)	8.32	0.0156	-	-	-	-
Microbial Respiration (biomass-specific)	6.54	0.0381	-	-	-	-
Microbial carbon use efficiency	15.68	0.0004	-	-	-	-

Table S2. Linear mixed effects model outputs for treatment (alpine plants, lowland plants) effects on soil variables in the glasshouse experiment.

Response variable	LR	P
Soil pore water absorbance (a_{350})	24.84	< 0.0001
Soil pore water total fluorescence (F_{tot})	27.72	< 0.0001
Soil pore water fluorescence index (FI)	9.55	0.0020
Microbial biomass carbon	0.16	0.6932
Fast-decaying soil carbon pool size	3.99	0.0458
Fast-decaying soil carbon pool decay rate	0.23	0.6306
Soil DOM C1 (protein-like)	21.39	< 0.0001
Soil DOM C2 (protein-like)	10.42	0.0012
Soil DOM C3 (humic-like)	15.65	0.0001
Soil DOM C4 (humic-like)	9.60	0.0019
Soil DOM C5 (humic-like)	17.39	< 0.0001
Soil DOM C6 (fulvic acid-like)	16.50	< 0.0001

Table S3. Statistical test outputs for lowland species identity effects on soil carbon loss.

Explanatory variable	Linear model test statistics		
	F	d.f.	P
<u>Western Alps experiment</u>			
<i>Achillea millefolium</i>	0.17	1,4	0.7007
<i>Bellis perennis</i>	0.40	1,4	0.5634
<i>Bromus erectus</i> *	-	-	-
<i>Dactylis glomerata</i> *	-	-	-
<i>Medicago lupulina</i> *	-	-	-
<i>Plantago media</i>	1.19	1,4	0.3364
<i>Salvia pratensis</i>	0.10	1,4	0.7684
<u>Central Alps experiment</u>			
<i>Brachypodium pinnatum</i>	3.70	1,8	0.3053
<i>Carex flacca</i>	28.24	1,8	0.1184
<i>Carum carvi</i>	5.94	1,8	0.2479
<i>Dactylis glomerata</i> *	-	-	-
<i>Hypericum perforatum</i>	10.11	1,8	0.1939
<i>Plantago lanceolata</i> *	-	-	-
<i>Primula veris</i>	6.41	1,8	0.2395
<i>Ranunculus bulbosus</i>	40.38	1,8	0.0994
<i>Salvia pratensis</i>	0.08	1,8	0.8281
<i>Silene vulgaris</i>	36.12	1,8	0.1050
<i>Trifolium montanum</i> *	-	-	-
<i>Viola hirta</i> *	-	-	-

* NB: species not tested, present in < 3 plots

796 **Table S4. DOM components identified through PARAFAC modelling.**

797

Name	Excitation λ (nm)	Emission Λ (nm)	General description [†]	Peak name [*]
C1	275	332	Protein-like (tryptophan)	T
C2	270	310	Protein-like (tyrosine)	B
C3	340	434	Humic-like	A/C
C4	305	424	Microbial humic-like	M
C5	375	452	Humic-like	C
C6	< 250 (400)	506	Soil fluvic acid-like	C+

^{*} Ref 57; [†] Ref 56

798