Lowland plant arrival in alpine ecosystems facilitates a decrease in soil carbon content under experimental climate warming

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Abstract

Climate warming is releasing carbon from soils around the world[1–3], constituting a positive climate feedback. Warming is also causing species to expand their ranges into new ecosystems[4–9]. Yet, in most ecosystems, whether range expanding species will amplify or buffer expected soil carbon loss is unknown[10]. Here we used two whole-community transplant experiments and a follow-up glasshouse experiment to determine whether the establishment of herbaceous lowland plants in alpine ecosystems influences soil carbon content under warming. We found that warming (transplantation to low elevation) led to a negligible decrease in alpine soil carbon content, but its effects became significant and 52% ± 31% (mean ± 95% CIs) larger after lowland plants were introduced at low density into the ecosystem. We present evidence that decreases in soil carbon content likely occurred *via* lowland plants increasing rates of root exudation, soil microbial respiration and CO_2 release under warming. Our findings suggest that warming-induced range expansions of herbaceous plants have the potential to alter climate feedbacks from this system, and that plant range expansions among herbaceous communities may be an overlooked mediator of warming effects on carbon dynamics.

Introduction

Climate warming is expected to accelerate the release of soil carbon to the atmosphere[11]. At the same time, warming is driving uphill and poleward range expansions of native plant species around the globe[4–9]. Yet while warming and plant range expansions are now co-occurring in many terrestrial ecosystems, the impact of range expanding plants on soil carbon storage under warming remains poorly understood[10]. This is important because plant species adapted to different temperatures can have contrasting effects on carbon cycle processes[12], raising the potential for migrating species to have qualitatively different impacts on carbon dynamics from resident plants. For example, studies of non-native species invasions have revealed that the arrival of novel species in a community can alter carbon cycling in recipient ecosystems[13,14], while woody plant expansions into treeless Arctic ecosystems can reverse the direction of carbon cycle feedbacks to climate warming[15,16]. However, the majority of warming-induced range expansions do not involve non-native species or novel growth forms, but occur among native communities of similar species (e.g. ref [9]). In particular, we know remarkably little about how migrations of herbaceous plants into predominantly herbaceous ecosystems will affect carbon cycling, and so the magnitude and direction of resulting climate feedbacks are unknown.

Alpine ecosystems occur in the uppermost elevations of the planet as cool islands within warmer landscapes that are bounded by steep gradients in temperature[17]. They are experiencing both rapid temperature change (0.4 to 0.6 ºC per decade)[18,19] and rapid species immigration[20], the latter of which involves an influx of herbaceous warm-adapted species from lowlands into herbaceous cold-adapted communities at higher elevations[7–9]. Coupled to this, alpine ecosystems are globally relevant reservoirs of soil carbon and hold more than 90% of their total ecosystem carbon in the soil[1–3,21,22]. As such, alpine ecosystems are not only themselves important for climate feedbacks, but are also sentinels for climate warming impacts on other terrestrial ecosystems. Here, we used alpine ecosystems as a model system to determine how the establishment of range expanding native plant species could affect soil carbon storage in warming ecosystems. We first used two whole-community transplant experiments in different alpine regions (western/central Swiss Alps) to establish the effects of warming plus lowland plant arrival on alpine soil carbon content. Second,

70 we used these experiments plus glasshouse and laboratory studies to explore potential
71 mechanisms through which lowland plants influence the alpine soil carbon cycle, testing the
72 hypothesis that they act *via* changes to root exudation[23,24].
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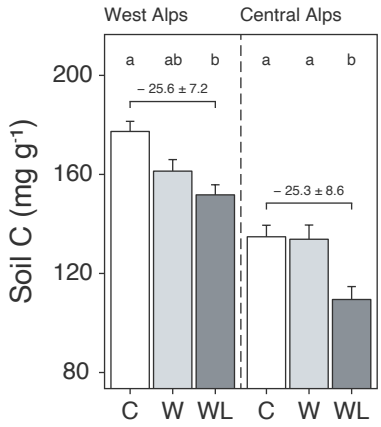

75
76
77 **Figure 1 | Warming and lowland plant effects on alpine soil carbon content in the field experiment.** Mean ± SE soil carbon
78 content (mg C g^{-1} dry mass; i.e. mass-based per-mil) in alpine turfs transplanted to low elevation (warming, W; light grey), transplanted
79 plus planted with lowland plants (warming plus lowland plant arrival, WL; dark grey) or replanted at high elevation (control, C; white).
80 Data are displayed for two experiments in the western (left) and central (right) Alps, with letters indicating treatment differences (LMEs;
81 N = 58).
82
83
84 **Results & Discussion**
85 Lowland plants facilitate warming-induced soil carbon loss
86 We exploited two independent field-based transplant experiments in which alpine plant
87 communities plus rooting zone soil were transplanted from high to low elevation to simulate a
88 climate warming scenario, or back into a high elevation site as a negative control (Supplementary
89 Fig. S1; Methods). A portion of the alpine turfs at low elevation were planted with local lowland
90 plant species to simulate the arrival of warm-adapted lowland plants in the warmed ecosystem,
91 with the remaining portion being subjected to a planting disturbance control. Elevation-based
92 transplant experiments are powerful tools for assessing climate warming effects on ecosystems
93 because they expose plots to a real-world future temperature regime with natural diurnal and
94 seasonal cycles while also warming both aboveground and belowground subsystems[25–27]. This is

95 especially true if they include rigorous disturbance controls (here, see Methods) and are performed
96 in multiple locations where the common change from high to low elevation is temperature (here,
97 warming of 2.8 ºC in the central Alps and 5.3 ºC in the western Alps). While factors other than
98 temperature can co-vary with elevation[17], such factors either do not vary consistently with elevation
99 among experiments (e.g. precipitation, wind), are not expected to strongly influence plant
100 performance (e.g. UV radiation) or in any case form part of a realistic climate warming scenario
101 (e.g. growing-season length, snow cover)[17,27,28]. In short, the experiments used here examined how
102 the arrival of warm-adapted lowland plants influences alpine ecosystems in a warmed climate
103 matching lowland site conditions (i.e. turf transplantation to low elevation plus lowland plant
104 addition) relative to warming-only (i.e. turf transplantation to low elevation) or control (i.e. turf
105 transplantation within high elevation) scenarios.
106
107 We found that transplantation from high to low elevation (i.e. warming-only) caused a small decline
108 in alpine soil carbon content, but in both regions this effect was stronger and only became
109 statistically significant when lowland plants were also planted-in (Fig. 1; Table S1; $P < 0.001$).
110 Specifically, considering field data from both experiments together, the addition of lowland plants to
111 transplanted alpine turfs increased warming-induced soil carbon loss from 4.1 ± 0.23 mg g^{-1} per ºC
112 to 6.3 mg g^{-1} per ºC, which is a 52% ± 31% (mean ± 95% confidence intervals) increase relative to
113 warming alone (Supplementary Fig. S3; $P = 0.001$). We caution against equating changes to soil
114 carbon content with changes to soil carbon stock in the absence of coupled measurements of soil
115 bulk density (Methods). Nevertheless, these findings show that once warm-adapted lowland plants
116 establish in warming alpine communities, they facilitate warming effects on soil carbon loss on a
117 per gram basis. We thus suggest that uphill migrations of lowland plants, which are already
118 globally common[8,20], may have a hitherto unrecognised bearing over alpine soil carbon storage.
119
120 <u>Lowland and alpine plants have distinct effects on soil microbial respiration</u>
121 Soil carbon loss occurs due to a shifting balance between soil carbon inputs *via* net primary
122 production and soil carbon outputs *via* soil microbial respiration[29,30]. We therefore hypothesised
123 that lowland plants impacted alpine soil carbon loss by either decreasing net primary production or

increasing soil microbial respiration relative to alpine plants. 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. The glasshouse experiment was not a direct simulation of field conditions, in that plants from lowland and alpine communities were grown separately at a constant temperature and humidity (Methods). Instead, the glasshouse experiment allowed us to isolate how plants adapted to lowland versus alpine climates differentially affect the alpine soil system.

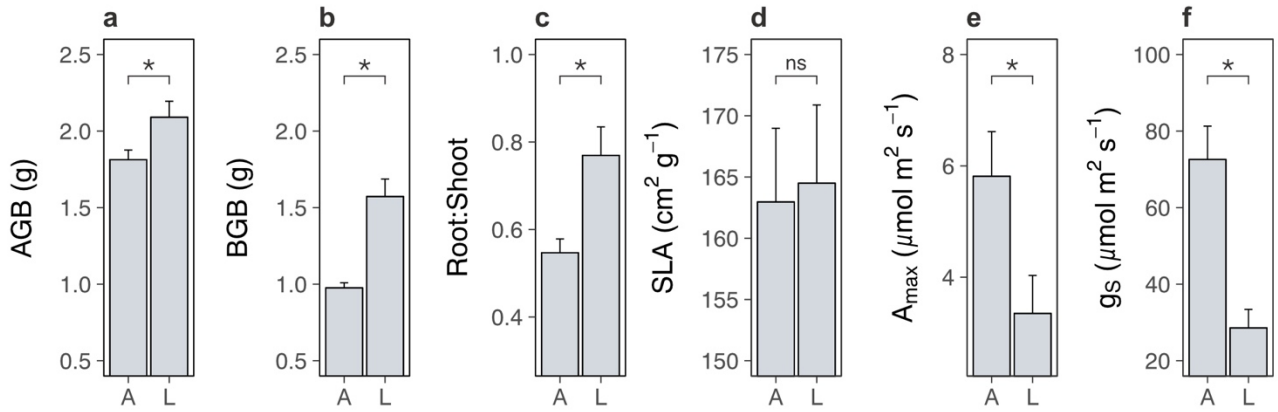

Fig. 2 | Differences between lowland and alpine plant traits in the glasshouse experiment. Mean ± SE pot-level traits for alpine (A) and lowland (L) plant treatments in a glasshouse experiment. (a) Aboveground biomass (AGB; g; N = 19). (b) Belowground biomass (BGB; g; N = 10). (c) Root-to-shoot ratio (N = 19). (d-f) Biomass-weighted (d) specific leaf area (SLA; cm^2 g^{-1}; N = 18), (e) maximum photosynthetic capacity (A_{max}; μmol m^2 s^{-1}; N = 19) and (f) maximum stomatal conductance (g_s; μmol m^2 s^{-1}; N = 20). Treatment effects (P < 0.05) are indicated by asterisks (LMEs; see Table S2).

We first compared lowland and alpine plant traits to test whether lowland plants decreased carbon inputs into alpine soil. We found that lowland plant treatments produced more aboveground (Fig. 2a; Table S2; P = 0.028) and belowground (Fig. 2b; Table S2; P < 0.001) biomass than alpine plants, while also containing a higher proportion of root tissue (root-to-shoot ratio: Fig. 2c; Table S2; P = 0.005). While biomass is an imperfect proxy for net primary production, these findings

point away from the hypothesis that lowland plants cause alpine soil carbon loss by reducing carbon inputs into soil. On the contrary, the higher biomass and proportionately more root tissue present in lowland plant treatments suggests that lowland plants may in fact increase soil carbon inputs. This occurred despite lowland plant treatments possessing similar specific leaf area (SLA: Fig. 2d; Table S2; $P = 0.861$) to alpine plant treatments, as well as lower maximal photosynthetic capacity (A_{max}: Fig. 2e; Table S2; $P = 0.026$) and stomatal conductance (g_s: Fig. 2f; Table S2; $P < 0.001$). While the reason for decreased photosynthetic capacity and stomatal conductance in the lowland plant treatment is unclear, the fact that both variables were lower suggests that photosynthetic capacity was limited by stomata pore size rather than by photosynthetic machinery[31]. Indeed, lowland plants accumulated more biomass despite having a lower maximal photosynthetic capacity, ruling it out as a limiting factor of lowland plant net primary production.

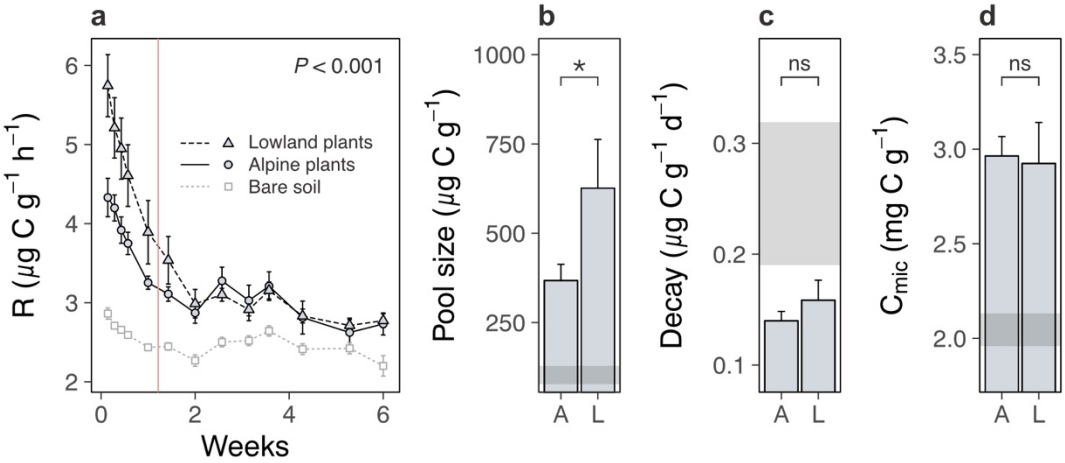

Fig. 3 | Lowland and alpine plant effects on alpine soil microbes in the glasshouse experiment. Effects of alpine (A) versus lowland (L) plant treatments on alpine soil microbes in a glasshouse experiment (mean ± SE; grey points/ribbons: SEs for bare soil). (a) Total microbial respiration (R; μg C g^{-1} dry mass h^{-1}) during follow-up incubations without plants (N = 260). (b) Size (μg C g^{-1} dry mass) and (c) decay rate (μg C g^{-1} dry mass d^{-1}) of a carbon pool identified using two-pool models (N = 16). (d) Microbial biomass carbon (C_{mic}; mg C g^{-1} dry mass; N = 18). For (a), vertical line indicates time before which A-L comparison differed (LME, Tukey; see Main Text). For (b-d), treatment effects ($P < 0.05$) are indicated by asterisks (LMEs; see Table S2).

171 We next examined the soil system under lowland versus alpine plants to determine whether
172 lowland plants alternatively increased carbon outputs from alpine soil. We found that lowland
173 plants accelerated soil microbial respiration relative to alpine plants (Fig. 3a; treatment × time:
174 likelihood ratio (LR) = 147.99, $P < 0.001$). Accelerated microbial activity was also reflected in
175 spectral analyses of soil pore water, which showed that soil under lowland plants contained more
176 dissolved organic matter (Fig. 4a,b; Table S2; absorbance (a_{350}): $P < 0.001$, total fluorescence
177 (F_{tot}): $P < 0.001$) with a higher proportion of microbially-derived products (Fig. 4c; Table S2; FI; $P =$
178 0.002) than soil under alpine plants. Importantly, microbial respiration was enhanced despite no
179 changes to microbial biomass between treatments (Fig. 3d; Table S2; $P = 0.693$), showing that
180 microbes respired intrinsically faster (i.e., per unit of biomass) in the presence of lowland plants.
181 These findings support the hypothesis that lowland plants have the capacity to increase soil carbon
182 outputs relative to alpine plants by stimulating soil microbial respiration and associated CO_2
183 release. While accelerated microbial respiration can alternatively be a signal of soil carbon
184 accumulation *via* greater microbial growth[30], such a mechanism is unlikely to have been
185 responsible here because it would have led to an increase in microbial biomass carbon under
186 lowland plants, which we did not observe.

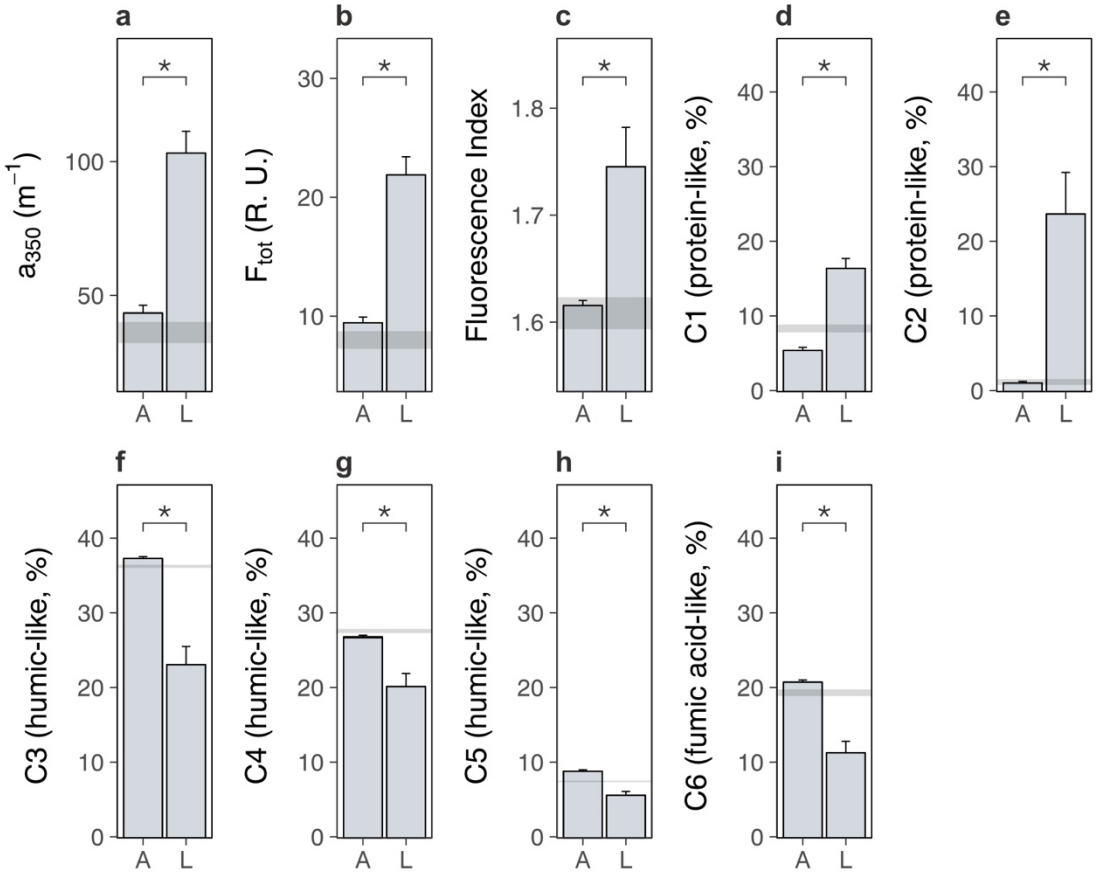

Fig. 4 | Lowland and alpine plants effects on alpine soil dissolved organic matter in the glasshouse experiment. Effects of alpine (A) versus lowland (L) plant treatments on alpine soil dissolved organic matter extracted from soil pore water in a glasshouse experiment (mean ± SE; grey ribbons: SEs for bare soil). (a) Absorbance (a_{350}; m^{-1}), (b) total fluorescence (F_{tot}; Raman Units) and (c) fluorescence index (FI) (N = 20). (d-i) Relative abundance (%) of six dissolved organic matter components (C1-C6), where components and associated compound families were derived from PARAFAC modelling (Methods; Table S4). Treatment effects ($P < 0.05$) are indicated by asterisks (LMEs; see Table S2).

We speculate that lowland plants accelerated microbial activity in the glasshouse experiment 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 a factor of 1.5 ± 0.7 (mean ± SE) times larger under lowland plants than alpine plants (Fig. 3b; Table S2; $P = 0.046$). This carbon pool decayed at a similar rate between treatments (Fig. 3c; Table S2; $P = 0.631$), indicating that it differed in size, but likely not composition, between lowland and alpine treatments. Second, the same carbon pool was consumed rapidly by soil microbes (half-life = 5.1 ± 0.4 days), and we

205 also found that lowland plant effects on microbial respiration were lost after nine days of incubation
206 without plants (Fig. 3a). Plants thus supplied compounds to microbes that were depleted within this
207 timeframe. While not directly measured here, a nine-day decay period corresponds to the time
208 expected for newly photosynthesised CO_2 to be released through root exudation and respired by
209 soil microbes[23,24], suggesting that this carbon pool was mostly root exudates. Third, lowland plant
210 effects on the alpine soil system were rapid, occurring within a period of weeks in the glasshouse
211 experiment and months in the field experiments. It is unlikely that lowland plants influenced
212 microbial activity *via* litter inputs within this timeframe[32,33]. Finally, dissolved organic matter
213 contained a higher relative abundance of protein-like substances than humic-like and fulvic acid-
214 like substances (Fig. 4d-i; Table S2; $P < 0.01$ in all cases) under lowland plants than alpine plants,
215 also suggesting that lowland plants caused an enrichment in exudate-derived compounds[34]. While
216 further directed studies are required to resolve whether root exudates are truly involved, our
217 findings collectively suggest that lowland plants have the capacity to increase total root exudation
218 into alpine soil relative to resident alpine plants. In turn, root exudation is a well-established
219 regulator of soil microbial decomposition and the associated release of soil carbon as CO_2[29,35].
220
221 One explanation for increased soil carbon outputs under lowland plants is that lowland plants have
222 more biomass than alpine plants and therefore stimulate microbial activity to a greater extent (Fig.
223 2a-c). We investigated this possibility by summarising pot-level traits from the glasshouse
224 experiment using a principal components analysis and testing for relationships between resulting
225 axes of trait variation and alpine soil microbial respiration (Methods). We found that descriptors of
226 plant biomass represented the most important axis of trait variation (Supplementary Fig. S4c) and
227 were positively related to microbial respiration (Supplementary Fig. S4a; PC1: LR = 4.43, N = 20, P
228 = 0.035). Thus, greater biomass of lowland plants may have contributed to amplified microbial
229 activity in soils with lowland plants in the glasshouse experiment. However, it is unlikely that
230 increased biomass was solely responsible for lowland plant effects in the field experiments
231 because lowland plant cover was low (mean ± SE relative cover: 4.7% ± 0.7%). Furthermore, in
232 the glasshouse experiment we found that trait variation represented by the second principal axis
233 had opposing effects on microbial respiration between lowland and alpine treatments

234 (Supplementary Fig. S4b; treatment × PC2: LR = 5.98, N = 20, P = 0.015). Lowland plants thus
235 displayed relationships with the alpine soil system that were qualitatively distinct from resident
236 alpine plants. While focussed work is now needed to pinpoint the physiological mechanism behind
237 these differences, the existence of such a distinction raises the possibility that the arrival of warm-
238 adapted lowland plants in warming alpine ecosystems initiates a change in plant community
239 functioning *via* changes to traits.
240
241 Plants adapted to different temperatures can possess distinct traits that have opposing effects on
242 carbon cycle processes[12]. We therefore tested whether lowland plants in both the glasshouse and
243 field experiments indeed differed from alpine plants in traits that could affect the alpine soil system.
244 Publicly available trait data[36] (plant height, leaf area, seed mass, SLA, leaf carbon, leaf nitrogen;
245 Methods) relating to all species in the field experiments revealed that lowland plants on average
246 possessed distinct traits from alpine plants (Supplementary Fig. S2a; P = 0.025). This was also the
247 case for measured traits of lowland versus alpine plants under standardised conditions in the
248 glasshouse experiment (multidimensional analyses: Supplementary Fig. S2b; P = 0.001). Coupled
249 to this, while lowland plants were present at low abundance in the field experiments (mean ± SE
250 relative cover: 4.7% ± 0.7%), the magnitude of soil carbon loss caused by lowland plant presence
251 was greater in plots where lowland plants were more abundant overall (Supplementary Fig. S2e;
252 $F_{1,14}$ = 5.79, P = 0.030). By contrast, we found no evidence that soil carbon loss in the field was
253 alternatively related to individual lowland species identity (Supplementary Fig. S2c,d) or the
254 composition of the recipient alpine plant community (Supplementary Fig. S2,g). Based on these
255 observations, we hypothesise that, at least in our experiments, the establishment of warm-adapted
256 lowland plants in warmed alpine ecosystems introduces novel traits into the community that alter
257 plant community functioning and thus carbon cycle processes, with effects that intensify as lowland
258 plants become more abundant in the community[32,33]. An alternative explanation is that, trait
259 differences aside, the ability of alpine plants to facilitate soil carbon loss is suppressed when
260 growing in warmer climates to which they are not adapted. While either explanation could
261 ultimately accelerate soil carbon loss from warming alpine ecosystems following the arrival of

262 lowland plants, future studies are needed to fully unravel the environmental-dependence of plant
263 trait effects on soil ecosystem processes.
264
265 Lowland plant-induced carbon loss is temporally dynamic
266 We have shown using two field experiments that lowland plants facilitate a decrease in soil carbon
267 content from warming alpine ecosystems (Fig. 1) and, with a glasshouse experiment, that they
268 potentially act by accelerating soil carbon outputs *via* microbial respiration relative to alpine plants
269 (Fig. 3, Fig. 4), as opposed to decreasing soil carbon inputs. We therefore hypothesised that
270 lowland plant arrival in transplanted alpine plots in the field experiments would drive a net release
271 of carbon dioxide from the ecosystem. As for the glasshouse experiment, we initially examined
272 how lowland plants affected the balance between carbon inputs and outputs of the ecosystem
273 using carbon dioxide flux measurements taken on multiple dates during the growing season
274 (Methods). We then used soil samples collected on one representative date to focus on intrinsic
275 rates (that is, per unit of biomass) of microbial respiration and growth as processes that promote
276 soil carbon loss and gain, respectively[29,30], as well as microbial biomass as the scalar through
277 which these processes affect soil carbon[37,38].
278
279 We found that transplantation from high to low elevation (i.e. warming-only) affected alpine
280 ecosystem net carbon dioxide exchange differently between regions (Fig. 5a; Table S1; $P = 0.040$).
281 In the western Alps, transplantation changed the ecosystem from a net sink to a net source of
282 carbon dioxide, whereas in the central Alps it made the ecosystem a stronger net carbon dioxide
283 sink. This effect occurred because although transplantation accelerated rates of both gross primary
284 production (Fig. 5b; Table S1; $P < 0.001$) and ecosystem respiration (Fig. 5c; Table S1; $P < 0.001$),
285 in the western Alps it had stronger effects on ecosystem respiration and in the central Alps
286 stronger effects on gross primary production. Importantly, lowland plants had no significant bearing
287 over net ecosystem exchange (Fig. 5a), implying that although lowland plants were associated with
288 soil carbon loss from warmed alpine plots (Fig. 1), this must have occurred prior to carbon dioxide
289 measurements being taken and was no longer actively occurring. Gross primary production in
290 warmed alpine plots was also unaffected by lowland plant presence (Fig. 5b), which is consistent

with the low abundance of lowland plants in alpine communities (mean ± SE relative cover: 4.7% ± 0.7%). As such, lowland plants likely did not facilitate alpine soil carbon loss under warming by decreasing carbon inputs into the ecosystem. By contrast, ecosystem respiration in warmed alpine plots was depressed in the presence versus absence of lowland plants (Fig. 5c). These findings generally support the hypothesis that lowland plants affect the alpine soil system by changing carbon outputs. However, they contrast with expectations that lowland plants perpetually increase carbon outputs from the ecosystem and thus raise questions about how soil carbon was lost from warmed plots containing lowland plants (Fig. 1).

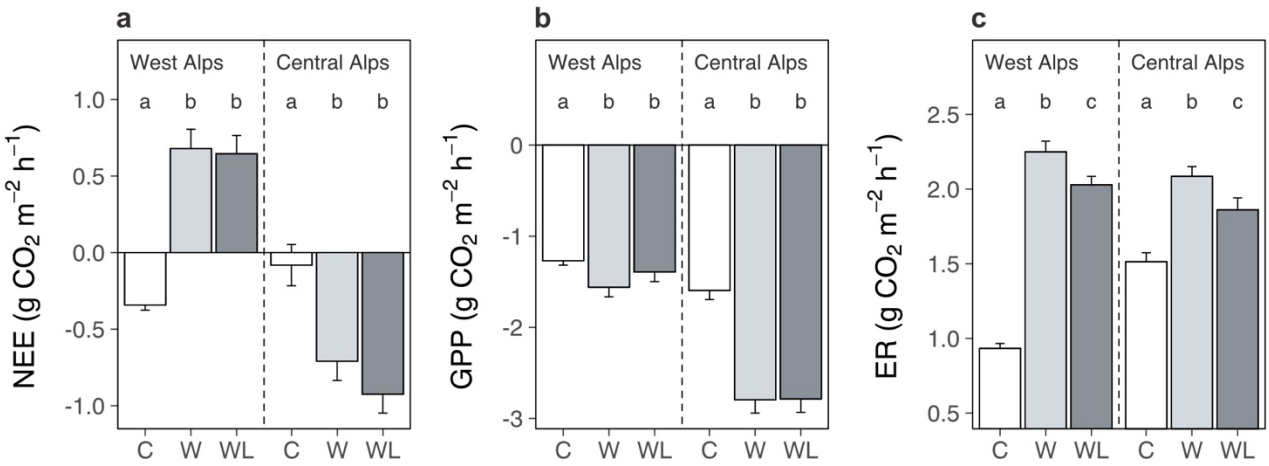

Fig. 5 | Warming and lowland plant effects on alpine ecosystem carbon dioxide fluxes in the field experiment. Effects of transplantation to low elevation (warming, W), transplantation plus lowland plants (warming plus lowlands, WL) or replantation at high elevation (control, C) on alpine ecosystem carbon dioxide fluxes (mean ± SE) during one season. (a) Net ecosystem CO_2 exchange (NEE; g CO_2 m^{-2} h^{-1}). (b) Gross primary production (GPP; g CO_2 m^{-2} h^{-1}). (c) Ecosystem respiration (ER; g CO_2 m^{-2} h^{-1}). Measurements were taken *in situ* at both experiments on multiple dates (western Alps: N = 196, central Alps: N = 90), with negative values indicating carbon uptake into the ecosystem. Treatment effects ($P < 0.05$) are indicated by letters (LMEs, Tukey; see Table S1).

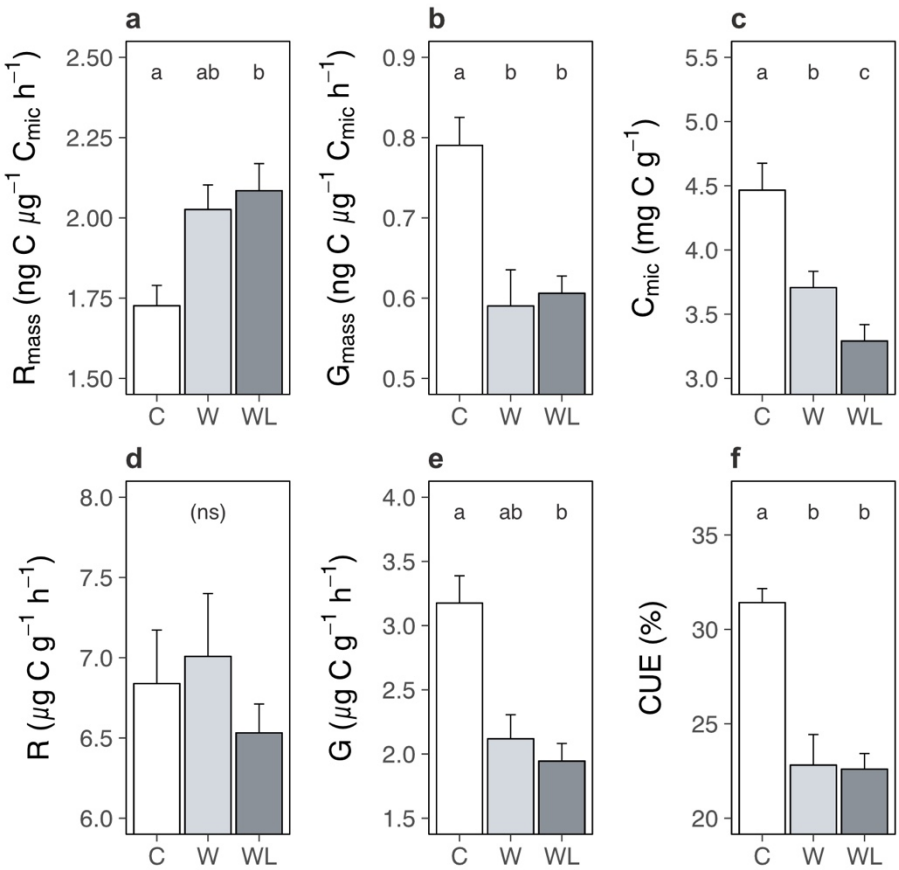

Fig. 6 | Warming and lowland plant effects on the alpine soil system in the field experiment. Effects of transplantation to low elevation (warming, W), transplantation plus lowland plants (warming plus lowlands, WL) or replantation at high elevation (control, C) on alpine soil pools and processes (means ± SEs) after one season. (a) Biomass-specific microbial respiration (R_{mass}; ng C μg^{-1} C_{mic} h^{-1}). (b) Biomass-specific microbial growth (G_{mass}; ng C μg^{-1} C_{mic} h^{-1}). (c) C_{mic} (μg C g^{-1}). (d) Total microbial respiration (μg C g^{-1} h^{-1}). (e) Total microbial growth (μg C g^{-1} h^{-1}). (f) Microbial carbon use efficiency (CUE; %). Measurements were taken on soils sampled on one date, for (f) at both experiments (western Alps: N = 28; central Alps: N = 30) and for all other variables at the western Alps experiment (N = 28). Treatment effects ($P < 0.05$) are indicated by letters (LMEs, Tukey; see Table S1).

Carbon cycle processes are constrained by multiple feedbacks within the soil system, such as substrate availability[37,38] and microbial acclimation[39–41], that over time can slow, or even arrest, soil carbon loss[38,40,41]. We thus interrogated the state of the soil system in the field experiments in the western Alps experiment to explore whether such a feedback may be operating here, in particular to limit ecosystem respiration once soil carbon content had decreased in warmed alpine plots containing lowland plants. We found that biomass-specific rates of microbial respiration in the field were greater following transplantation to the warmer site in the presence, but not absence, of

327 lowland plants (Fig. 6a; Table S1; $P = 0.038$). By contrast, biomass-specific rates of microbial
328 growth were slower following transplantation but remained unaffected by lowland plant presence
329 (Fig. 6b; Table S1; $P = 0.016$). Thus, despite lower rates of ecosystem respiration overall, alpine
330 soil microbes still respired intrinsically faster in warmed plots containing lowland plants. Moreover,
331 accelerated microbial respiration, but not growth, implies that alpine soils had a higher capacity to
332 lose carbon under warming, but not to gain carbon *via* accumulation into microbial biomass, when
333 lowland plants were present[29,30]. These findings align with observations from the glasshouse
334 experiment that lowland plants generally accelerated intrinsic rates of microbial respiration (Fig. 3),
335 although in field conditions this effect occurred in tandem with warming.
336
337 Faster microbial respiration can only yield a loss of soil carbon if it occurs on a per unit of soil
338 basis[38,41,42], which was the case in the glasshouse experiment (Fig. 3). However, in the field we
339 found that lowland plants caused a reduction of microbial biomass carbon in alpine communities
340 transplanted to the warmer site (Fig. 6c; Table S1; $P < 0.001$). As such, although microbes
341 generally respired intrinsically faster in the presence of lowland plants, in the field they had
342 become less abundant and so we observed no change to microbial respiration when expressed
343 per unit of soil (Fig. 6d; Table S1; $P = 0.550$). The decline in microbial biomass likely took place
344 because soil microbes are chiefly carbon limited[43], making their abundance tightly coupled to soil
345 carbon content (here: $r_{26} = 0.61$, $P = 0.001$)[38]. Moreover, since intrinsic rates of microbial growth
346 were unaffected by lowland plant presence, a reduction in microbial biomass translated into a
347 decline in microbial growth per unit of soil (Fig. 6e; Table S1; Table S1; $P = 0.009$) – preventing
348 any recovery of microbial biomass *via* growth[30]. We thus suggest that as soil carbon was lost,
349 microbial biomass also declined, and at the time of measurement this had eliminated the influence
350 of accelerated biomass-specific microbial respiration over the soil system. In short, we propose
351 based on these relationships that the decrease in soil carbon content observed in warmer
352 transplanted plots containing lowland plants had both started and stopped within one season.
353
354 Taken together, one interpretation of our findings is that the establishment of lowland plants in
355 warming alpine ecosystems accelerates intrinsic rates of microbial respiration (Fig. 3, Fig. 6a),

leading to soil carbon release at baseline levels of microbial biomass (Fig. 1, Fig. 3c), a coupled decline in microbial biomass (Fig. 6c) and a cessation of further carbon loss from the ecosystem (Fig. 5a, Fig. 6d). Although such a mechanism has been reported in other ecosystems[38,41], applying it here is speculative without additional timepoints because field soil measurements came from a single sampling event after soil carbon had already been lost from the ecosystem. For instance, an alternative mechanism could be that soil microbes acclimate to the presence of lowland plants and this decelerated microbial processes over time[39–41]. Nevertheless, it remains true that biomass-specific microbial growth and respiration affect the soil system as a function of microbial biomass, and here changes to microbial biomass could 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. 6f; Table S1; $P < 0.001$; Tukey: $P = 0.996$), pointing away from microbial acclimation as an alternative mechanism. Beyond the mechanism for lowland plant effects on alpine soil carbon loss, it is conceivable that soil carbon loss is not isolated to a single season, but will reoccur in the future even without further warming or lowland plant arrival. This is especially true in the western Alps experiment where warming yielded a net output of carbon dioxide from the ecosystem (Fig. 5a). Moreover, in our field experiments we simulated a single event of lowland plant establishment and at relatively low abundance in the community (mean ± SE relative cover: 4.7% ± 0.7%), raising the possibility that increases in lowland plant cover or repeated establishment events in the future could facilitate further decreases in alpine soil carbon content under warming.

General conclusions

In summary, we show that uphill migrations of native lowland plants have the potential to facilitate warming effects on soil carbon loss from alpine ecosystems. This is important because it demonstrates that plant range expansions between herbaceous plant communities have the potential to contribute significantly to climate feedbacks. These results contrast with expectations from woody species expansions in the Arctic, where shrubs with recalcitrant litter can slow carbon turnover in herbaceous communities and potentially yield a negative climate feedback[15,16]. We thus

suggest that the influence of range expanding species on ecosystem carbon dynamics depends on the relative trait composition of migrating species versus the recipient community[44], as well as subsequent effects on environmental processes[15]. While our findings demonstrate that lowland plants affect the rate of soil carbon release in the short term, short-term experiments, such as ours, cannot resolve whether lowland plants will also affect the total amount of soil carbon lost in the long term. This includes whether processes such as genetic adaptation (in both alpine and lowland plants)[12] or community change[45,46] will moderate soil carbon responses to gradual or sustained warming. Nevertheless, here decreases in soil carbon content occurred rapidly despite a low abundance of lowland plants, and increased with total lowland plant cover. Given that both warming and associated plant migrations will intensify over time[8,9], our estimates of lowland plant impacts may be conservative – particularly if increases in lowland plant cover or repeated establishment events cause recurrent pulses of soil carbon release. Moreover, while alpine ecosystems store only a small fraction of total global soil carbon[1,2], redistributions among herbaceous plant communities are occurring globally[4–9], including in similarly cool ecosystems in the Arctic that contain vast soil carbon stocks[47]. This raises the potential for resulting climate feedbacks to also be widespread. Future work should focus on testing the conditions under which this feedback could occur in different mountain regions, as well as other ecosystems, experiencing influxes of range expanding plant species, on quantifying how deeply it occurs in shallow alpine soils, and on estimating the magnitude of the climate feedback given both ongoing warming and variation in rates of species range shifts.

405 **Materials & Methods**

406 **Field site description, experimental design.** We made use of two independent alpine grassland
407 transplant experiments that were established in Switzerland in the western Alps (46°12′40″N,
408 7°03′13″E)[28] and central Alps (46°52′20″N, 9°28′58″E) in autumn 2016. The experiments simulate
409 effects of climate warming on alpine ecosystems *via* transplantation from high to low elevation,
410 including the arrival of lowland plants due to warming-induced uphill plant migrations
411 (Supplementary Fig. S1). In the western Alps experiment the plant community is dominated by
412 *Sesleria caerulea*, *Carex sempervirens* and *Vaccinium vitis-idea*. In the central Alps experiment the
413 dominant species are *Nardus stricta*, *Alchemilla xanthochlora*, *Trifolium pratense* and *Anthyllis*
414 *vulneraria*. Broadly, the experiments share the same design. Turfs (area = 1 m^2, depth = 20 cm) of
415 intact alpine vegetation plus rhizosphere soil were removed from a high elevation control site
416 (western Alps: 2200 m, MAT = 0.7 ºC, TAP = 1448 mm; central Alps: 2000 m, MAT = 1.6 ºC, TAP
417 = 1151 mm; ref. [48]) and replanted either at a low elevation site to simulate climate warming
418 (western Alps: 1400 m, MAT = 6.0 ºC, TAP = 1236 mm, N = 10; central Alps: 1400 m, MAT = 4.4
419 ºC, TAP = 1010 mm, N = 20; ref. [48]) or back into a high elevation site as a transplantation control
420 (see below). At the low elevation site, we created an additional treatment to simulate the arrival of
421 lowland plants under warming by planting a low abundance of lowland plant species in a grid
422 design (13 cm between individuals), creating a lowland plant density of approximately 56
423 individuals per m^2 (4.7% ± 0.7% total cover) in a background alpine plant community. Lowland
424 plants were positioned randomly in the grid, but with the same number of individuals per species
425 per plot, and we controlled for planting disturbance in all remaining plots using alpine plants in
426 place of lowland plants (see below). In the western Alps region, lowland plant species were
427 *Achillea millefolium, Bellis perennis, Bromus erectus, Dactylis glomerata, Medicago lupulina,*
428 *Plantago media* and *Salvia pratensis*, and individuals were planted into one side of each plot in a
429 split-plot design (n = 10). In the central Alps experiment, lowland plant species were *Brachypodium*
430 *pinnatum, Carex flacca, Carum carvi, Dactylis glomerata, Hypericum perforatum, Plantago*
431 *lanceolata*, *Primula veris, Ranunculus bulbosus, Salvia pratensis*, *Silene vulgaris*, *Trifolium*
432 *montanum* and *Viola hirta*, and individuals were planted into half of the transplanted turfs (n = 10).
433 While exact dispersal distances for selected lowland species are unknown, all species are

434 widespread and are expected to migrate uphill under warming[8] and the horizontal distance
435 between high and low sites in the field experiments was always less than 2 km. In the western Alps
436 experiment, lowland individuals were pre-grown in a greenhouse in steam-sterilized field soil and
437 planted into the plots in May 2017. In the central Alps experiment, lowland individuals were taken
438 from the surrounding vegetation and planted into the plots in autumn 2016. In both regions, soil
439 was partially removed from lowland individuals prior to planting, and we performed an additional
440 experiment to eliminate the possibility that small contaminations of lowland soil biota during
441 planting could have modified alpine soil microbial community composition (see *Field Experiment*
442 *Soil Biota Validation*, below). Overall, the experimental design in both regions yielded three
443 treatments (n = 10 for each) arranged in a block design: transplantation from high to low elevation
444 (i.e. warming-only, W); transplantation plus low elevation plants (i.e. warming plus lowland plant
445 establishment, WL); and replantation at the high elevation origin site (i.e. control, C). One turf at
446 the low elevation site in the western Alps experiment failed, leaving a total sample size of N = 28
447 and N = 30 in the western Alps and central Alps, respectively.
448
449 **Field experiment controls and validation.** We controlled for transplantation disturbance in both
450 experiments by moving alpine control turfs back into a high elevation control site. In the western
451 Alps experiment, alpine turfs were planted into different positions at their origin high elevation site.
452 In the central Alps experiment, alpine turfs were planted into a new high elevation site at the same
453 elevation. As such, we eliminated the possibility that alpine turfs changed simply because they
454 were disturbed or were moved to a different location (that is, irrespective of elevation). The
455 transplantation process had no effect on alpine soil carbon content, in that we found no difference
456 in soil carbon content between replanted alpine control turfs at high elevation sites and undisturbed
457 alpine soil in the surrounding area (LR = 2.27, N = 16, P = 0.132). We also controlled for potential
458 effects of planting disturbance in all turfs not planted with lowland plants (that is, remaining turfs at
459 low elevation and all control turfs at high elevation). In the western Alps experiment, we planted
460 alpine species from the high elevation control site into the other side of alpine turfs at the low
461 elevation site, as well as into all control turfs at the high elevation site. This was done in autumn
462 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[35,49,50], 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. Finally, 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 [51,52]). We then added 3 L of inoculum to one half of each of 10 x 1 m^2 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 (ø = 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 fungi[53]. DNA was extracted

492 (NucleoSpin Soil kit, Macherey-Nagel, Düren, Germany) and amplified using quadruplicate PCRs,
493 alongside extraction and PCR blank controls. PCR products were purified (MinEluteTM PCR
494 purification kit, Qiagen, Hilden, Germany) prior to sequencing on a MiSeq sequencing platform
495 (Illumina, San Diego, USA). Sequence data were processed using the OBITools software
496 package[54] and following[55]. Paired-end reads were merged, assigned to their respective samples
497 and dereplicated, and sequences with too short read lengths and with only one read were
498 excluded. Remaining sequences were clustered using the SUMACLUST[56] algorithm and
499 summarized into operational taxonomic units (OTUs) using the Infomap Community Detection
500 Algorithm[57], following which we calculated per-sample relative OTU abundances for
501 bacteria/archaea and fungi separately. We found that the alpine soil microbial community was
502 resistant to the introduction of low elevation soil biota, in that inoculation had no effect on soil
503 bacterial (Supplementary Fig. S5a; PERMANOVA; $F_{1,9} = 0.75$, $P = 0.573$) or fungal
504 (Supplementary Fig. S5b; $F_{1,9} = 0.75$, $P = 0.774$) community composition. The inoculation
505 represented a strong soil biota treatment relative to contamination by hitchhiking microbes. As
506 such, even if some contamination by low elevation soil biota did occur, it was unlikely to have
507 resulted in a change to the extant soil community, ruling out the possibility that contamination was
508 alternatively responsible for the soil carbon loss observed when lowland plants were present in
509 transplanted alpine plots.
510
511 **Field plant community composition.** We surveyed the composition of alpine communities in
512 summer 2017. This was done by dividing each plot into 5 × 5 cm cells and visually estimating the
513 canopy cover of all species rooted in every grid cell. Species cover was approximated with an
514 ordinal scale in which the first three cover categories correspond to 1/16, 1/8, and 1/4 of the grid
515 cell area (25 cm^2), and subsequent cover categories were constant increases of 1/4 of the grid cell
516 area. Plants were identified to species level whenever possible, although some taxa were identified
517 only to genus or subfamily levels when species identity was unclear based on vegetative traits.
518 Species cover (cm^2) was then calculated as the species-wise sum of cover estimates across all
519 grid cells in the quarter of the plot used for soil and ecosystem respiration measurements (see
520 below).

521

522 **Field soil carbon content, microbial physiology, microbial biomass.** We collected soil
523 samples from all treatments in both field experiments (N = 58) in August 2017. In each plot, we
524 created a composite sample from three cores (ø = 1 cm, approx. *d* = 7 cm) no closer than 7 cm
525 from a planted individual and from the same quarter of the plot used for ecosystem respiration
526 measurements (see below; Supplementary Fig. S1). We focused on rhizosphere soil for two
527 reasons. First, alpine soils are shallow and alpine plants are shallow-rooting, making the
528 rhizosphere the most logical target for a first assessment of warming and lowland plant effects on
529 alpine soil carbon cycling. Second, we wanted to avoid any potential contamination from the base
530 of the turf (depth = 20 cm). Soils were kept cool, transported immediately to the laboratory, sieved
531 (2 mm mesh size), adjusted to 60% of water holding capacity (WHC) and pre-incubated for three
532 days at field temperature prior to measurements. We measured microbial biomass carbon (mg per
533 g dry mass) *via* chloroform fumigation extraction followed by analysis on a TOC-VCPH/CPNTNM-1
534 analyzer (Shimadzu; 48h incubation period; 1 M KCl extraction for fumigated and non-fumigated
535 samples; conversion factor 0.45)[58]. While this approach also yielded measurements of dissolved
536 organic carbon (DOC; mg C per g dry mass), treatment effects on this variable echoed those of
537 microbial biomass carbon (Table S1) so we did not interpret them separately. Soil organic carbon
538 content (%) was measured on dry soil (60 ºC for 48h) with a Carlo Erba 1110 elemental analyzer
539 (EA, CE Instruments) coupled to a Delta Plus IRMS via a Conflo III (Thermo Fisher). It was not
540 possible to take widespread measurements of soil bulk density due to the destructive sampling
541 required while other studies were underway (e.g. ref [28]). Instead, we took additional soil cores (ø =
542 5 cm, *d* = 5 cm) from the central Alps experiment in 2021 once other studies were complete to
543 indirectly explore whether lowland plant effects on soil carbon content in warmed alpine plots could
544 have occurred due to changes in soil bulk density. We found that although transplantation to the
545 warmer site increased alpine soil bulk density (LR = 7.18, $P = 0.028$, Tukey: $P < 0.05$), lowland
546 plants had no effect (Tukey: $P = 0.999$). It is not possible to make direct inferences about the soil
547 carbon stock using measurements made on different soil cores four years apart. Nevertheless,
548 these results make it unlikely that lowland plant effects on soil carbon content in warmed alpine
549 plots occurred simply due to a change in soil bulk density. We additionally quantified microbial

growth, respiration and carbon use efficiency in soils from the western Alps experiment (N = 28) using the incorporation of ^{18}O into DNA[38]. Briefly, we incubated 500 mg of soil for 24h at field temperature following addition of either ^{18}O-H_2O to 20 atom% enrichment and 80% of WHC or the same volume of molecular grade non-labelled H_2O as a natural abundance control. Gas samples were taken at the start and end of the 24h incubation and analyzed for CO_2 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 ^{18}O abundance and total oxygen content using a thermochemical EA coupled to a Delta V Advantage IRMS via a Conflo III (Thermo Fisher). Following ref. [38], 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 (%).

Field ecosystem respiration. We measured rates of ecosystem respiration (g CO_2 per m^2 per h) on multiple dates (western Alps: 7 dates; central Alps: 3 dates) between July and August 2017. One month prior to the first measurement, we installed permanent gas sampling collars (*h* = 10 cm, ø = 30 cm) in each plot to a depth of 5 cm to create an airtight seal at the soil surface[12]. Measurements were made by enclosing plants and soil within the collars with an opaque chamber (*h* = 50 cm, ø = 30 cm) and, following a 30s equilibration period, monitoring the increase in CO_2 concentration at 5s intervals over a 60s period (Vaisala GMP343 CO_2 probe attached to an HM70 meter, Vaisala). Chambers contained a fan to mix the headspace during measurements and a small sealable hole on the top that was opened when placing the chamber on sampling collars to avoid pressurization inside the chamber. Soil temperature and moisture were recorded for each measurement and used alongside measurements of air temperature, air pressure, enclosure volume and soil surface area to calculate the CO_2 flux[12]. Where necessary, we repeated measurements in the field to attain a visually consistent CO_2 change over time, and during data processing we discarded fluxes with an *r*-squared of less than 0.9 for this relationship.

579 **Glasshouse experiment design.** We established a six-week pot experiment to test for effects of
580 lowland versus alpine plants on the alpine soil system. Specifically, we filled pots (ø = 10 cm, depth
581 = 9 cm) with sieved soil (mesh size 4 mm) from the high elevation site of the western Alps field
582 experiment, and planted them with six lowland species (*Bromus erectus*, *Salvia pratensis*,
583 *Medicago lupulina*) or six alpine species (*Phleum alpinum*, *Anthyllis vulneraria* ssp. *alpestris*,
584 *Plantago alpina*) to create a lowland only treatment, an alpine only treatment and a bare soil
585 control (N = 30). We selected a grass, herb and legume species for each treatment, all of which
586 were found naturally at the low (lowland treatment) or high (alpine treatment) elevation sites in the
587 western Alps field experiment. Vegetated treatments contained two individuals of all lowland or
588 alpine species planted in a circular pattern such they were never adjacent to conspecifics or plants
589 of the same functional type (i.e. grass, herb, legume), making replicates within treatments identical
590 in species composition and neighbourhoods. The original design also included a mixed community
591 treatment containing one individual of each lowland and alpine species. However, the mixed
592 treatment had double the species richness of other vegetated treatments and contained an equal
593 cover of lowland and alpine plants (compared to < 5% lowland plant cover in field plots), so we
594 focussed our analyses on the comparison between alpine and lowland plant treatments to
595 understand how lowland plants affect the soil differently from alpine plants. All plants were pre-
596 grown from seed provided by commercial suppliers (www.hauenstein.ch, www.schutzfilisur.ch,
597 www.saatbau.at) in steam-sterilized potting soil for two to three weeks to approximately the same
598 biomass and root-washed immediately prior to planting. Pots were arranged in a randomized block
599 design (n = 10) in a greenhouse under standardized growing conditions (22 ºC; 50% humidity; no
600 artificial light), and were watered every two to three days as needed. After six weeks, we
601 destructively harvested the plants and soil to characterize treatment effects on plant physiology
602 and the soil system.
603
604 **Glasshouse plant physiology.** We measured maximum leaf-level photosynthesis (photosynthetic
605 capacity; A_{max}; µmol CO_2 per m^2 per s) and stomatal conductance (g_s; µmol H_2O per m^2 per s)
606 under optimum conditions on one individual per species per pot using an open path infrared gas
607 analyzer connected to a 2.5 cm chamber (CIRAS-2 and PLC-6, PP Systems)[12]. Readings were

608 taken once for each individual and corrected using measurements of leaf area (cm^2) on the same
609 leaf, which we also dried and weighed to determine specific leaf area (SLA; cm^2 per gram).
610 Following soil pore water sampling (below), we quantified total, aboveground and belowground
611 biomass of all individuals in the experiment by destructively sampling, washing, drying (60 ºC for
612 48h) and weighing all plant tissue.
613
614 **Glasshouse soil pore water analysis.** We collected soil pore water from all pots prior to soil
615 sampling (see below) to determine treatment effects on dissolved organic matter (DOM) quality.
616 Soil water was sampled by leaching pots twice with 50 mL sterile tap water, combining and filtering
617 leached water (filter pore size 0.2 µm) and measuring absorbance (200-900 nm with a 1nm
618 increment; 1 cm quartz cuvette; UV-vis spectrophotometer, Shimadzu) and fluorescence intensity
619 (excitation: 240-450 nm with a 5 nm increment; emission: 290-550 nm with a 2.5 nm increment;
620 Fluorolog-3 spectrometer, Horiba). We corrected absorbance spectra against a milli-Q water blank,
621 calculated Napierian absorption coefficients and used these data to derive absorbance at 350 nm
622 (a_{350}; a proxy for DOM content). We expressed fluorescence data as total fluorescence (F_{tot};
623 Raman Units) and the fluorescence index (FI), i.e. the ratio of emission intensity at 470 nm versus
624 520 nm at an excitation wavelength of 370 nm (a proxy for microbially-derived DOM[59]). We also
625 derived excitation-emission matrices (EEMs) to quantify the relative abundance of different
626 components of DOM through PARAFAC algorithms[60]. Following preprocessing (i.e. Raman
627 scattering removal and standardization to Raman Units) and normalization[61], we used the drEEM
628 toolbox[62] in MATLAB (Mathworks) to obtain a six-component PARAFAC model. The model was
629 validated with split-half analysis, random initialization and visualization of EEM residuals, after
630 which we calculated the relative abundance of each component as the maximum fluorescence
631 signal of the component against the total maximum fluorescence signal of all components. Finally,
632 we classified components against classical EEM nomenclature (Table S4; refs [63,64]). While
633 PARAFAC modelling provides a means to discriminate between DOM sources (e.g. components
634 C3 and C4; Table S4), we did not physically separate plant- and microbe-derived DOM when
635 sampling soil pore water. As such, observed changes to DOM composition were likely a result of
636 changes to both plant and microbial processes (see Main Text).

637

638 **Glasshouse soil carbon pools, microbial respiration.** We took soil cores (ø = 1 cm, *d* = 7 cm)
639 from the center of each pot (i.e. equidistant from all individuals) to quantify soil carbon pools and
640 microbial respiration. Soils were sieved (2 mm mesh size), adjusted to 60% of their WHC and pre-
641 incubated at 22 ºC for three days prior to measurements. We quantified concentrations of microbial
642 biomass carbon in all soils as for field soil (see above; mg C per g dry mass). We determined
643 microbial respiration by incubating 8 g sieved soil in the dark at 22 ºC for a further six-week period
644 without plants. We measured microbial respiration daily for the first four days, then approximately
645 biweekly until the end of the six-week period. We measured CO_2 concentrations (EGM-4, PP
646 Systems) of gas samples taken approximately 24h after flushing enclosed vials (*v* = 126 mL) with
647 CO_2-free air, and calculated microbial respiration (µg C per g dry mass per h) using accompanying
648 measurements of enclosure volume, air pressure and soil mass[12]. After each measurement, soils
649 were weighed and, when necessary, re-adjusted to 60% of their WHC.

650

651 **Two-pool mixed models of soil carbon mineralization.** We described the kinetics of soil carbon
652 mineralization during the follow-up incubation of glasshouse soil by employing a two-pool mixed
653 model to microbial respiration data[65]. Specifically, we constructed a model that considered a first
654 pool that followed first-order decomposition kinetics (i.e. a small soil carbon pool with a fast
655 turnover) and a second pool that followed zero-order kinetics (i.e. decay rate was constant over the
656 incubation period, representing a large soil carbon pool with a slow turnover):

657

658 $$C_{min} = C_l\left(1 - e^{-tk_l}\right) + B \cdot t$$

659

660 where C_{min} is the cumulative CO_2 respired (µg C g^{-1}) over the incubation time *t* (d), C_l and k_l are the
661 size (µg C g^{-1}) and decomposition constant (d^{-1}) of the first-order kinetics carbon pool, respectively,
662 and *B* is the basal respiration rate (µg C g^{-1} d^{-1}) of the zero-order kinetics carbon pool. Given that
663 C_{min} and *t* were known, we ran the two-pool mixed model with the nlsList function (maxiter = 100,
664 tol = 1 × 10^{-5}, minFactor = 1/1024) in R[66] using the package "nlme"[67] for each pot separately to

665 estimate values for C_l, k_l and B. Models failed to converge for one sample, and we excluded four
666 additional samples for which models made negative pool estimates (N = 25).
667
668 **Statistical analysis.** The majority of statistical test outputs appear as *P*-values in the Main Text,
669 with full outputs being provided in Tables S1 to S3. See text below for exceptions. Data analyses
670 and visualization were undertaken in R[66] using the packages "cowplot"[68], "drake"[69], "emmeans"[70],
671 "nlme"[67], "tidyverse"[71] and "vegan"[72]. Where necessary, region was accounted for in statistical
672 models as a fixed effect, since two levels was too few to be included as a random intercept term.
673 As such, region effects are presented (Tables S1, S3), but not interpreted. Statistical methods are
674 described as they appear in the Main Text.
675
676 Warming and warming plus lowland plant effects on soil carbon content: we used field data to test
677 for treatment (C, W, WL) effects on soil carbon content using an LME model including region as a
678 fixed effect and block as a random intercept term (see Table S1 for test output). We also
679 calculated how much more soil carbon loss occurred in the presence versus absence of lowland
680 plants by fitting a single LME testing for effects of soil temperature (plot means of all sampling
681 dates), the presence or absence of lowland plants and their interaction on soil carbon content (see
682 Supplementary Fig. S2 for test outputs). In order to compare slopes between C-W and C-WL
683 treatment combinations, we duplicated C treatment values, assigned one set to each C-W and C-
684 WL pair and accounted for this using a random intercept term. We also included region as a
685 random slope term. We then tested how much more soil carbon loss would occur per ºC of
686 warming when lowland plants are present by calculating the relative difference (% ± 95%
687 confidence intervals) between the C-WL and C-W slopes.
688
689 Plant mechanisms for soil carbon loss: we expressed soil carbon content as the block-wise percent
690 changes between WL and W treatments to derive a metric for lowland plant induced soil carbon
691 loss (i.e. % soil carbon remaining). We tested for lowland species identity effects on soil carbon
692 remaining using separate LMEs for each region (due to different lowland species pools) that tested
693 for individual species cover (cm^2) effects on soil carbon remaining in WL plots, including species as

694 a random intercept term (see Supplementary Fig. S2 for test outputs). We also performed separate
695 linear models including species as fixed effects, none of which were statistically significant (Table
696 S3). We tested for effects of total lowland species cover (cm^2) on soil carbon remaining in WL plots
697 using a linear model also including region as a fixed effect. We summarized alpine community
698 composition in W and WL treatments using an NMDS (Hellinger distance matrix, two axes),
699 following which we expressed NMDS scores as the block-wise percent changes between W and
700 WL treatments to derive metrics for lowland plant induced alpine plant community change, and
701 used a linear model that tested for effects of changes to NMDS scores on soil carbon remaining in
702 WL plots including region as a fixed effect (see Supplementary Fig. S2 for test outputs). For
703 reference, raw NMDS scores did not significantly differ between W and WL treatments (LME:
704 NMDS #1: LR = 1.57, P = 0.210; NMDS #2: LR = 2.67, P = 0.102). Finally, we tested for
705 differences between alpine and lowland species traits in the field experiment using a
706 PERMANOVA on a principal components analysis (PCA) of species mean trait values ($\log_{10}$ plant
707 height, $\log_{10}$ leaf area, $\log_{10}$ seed mass, SLA, leaf carbon, leaf nitrogen) from the TRY database[36]
708 for all species present (where necessary, missing values were gap-filled following[73]), and in the
709 glasshouse experiment using a PERMANOVA on a PCA of physiological measurements (total
710 biomass, leaf biomass, root biomass, SLA, A_{max}, g_s) for all plants (see Supplementary Fig. S2 for
711 test outputs). Traits were selected to explore broad life history differences between alpine and
712 lowland plant species[74], but were not used to identify the specific trait(s) responsible for lowland
713 plant effects on alpine soil carbon loss. We used TRY traits for field species due to the large
714 number of species present in the communities (N = 118), but used measured traits in the
715 glasshouse experiment to provide direct evidence of physiological differences between alpine and
716 lowland species.
717
718 Lowland versus alpine plant effects on the soil system: we used the glasshouse experiment to test
719 for lowland versus alpine plant treatment effects on soil microbial biomass carbon, the size (C_l) and
720 decay rate (k_l) of the first modelled soil carbon pool and all variables relating to soil DOM quality
721 using LMEs including block as a random intercept term. We tested for effects of treatment, time
722 and their interaction on soil microbial respiration using the same LME structure but including the

723 bare soil treatment. In all cases, including/excluding the bare soil treatment had no effect over
724 model outcomes, but mean ± SE values for this treatment are included in figures for reference.
725 Finally, we explored how plant physiology in lowland and alpine treatments at the pot level affected
726 initial rates of soil microbial respiration. We defined initial microbial respiration as the pot-wise
727 mean respiration rates of the first nine days of soil incubation (that is, while treatment effects
728 persisted; see Fig. 3a), and expressed pot-level plant physiology using scores from the first two
729 axes of a PCA performed on sums of total, root and shoot biomass and biomass-weighted means
730 of SLA, A_{max} and g_s. We then tested for effects of treatment, PC1 and PC2 scores and their
731 interaction on initial microbial respiration using an LME including block as a random intercept term.
732 Statistical test outputs for LMEs are provided in Table S2, except for treatment and plant
733 physiology effects on microbial respiration, which appear in the Main Text.
734
735 Sequence of warming and lowland plant effects on alpine soil carbon loss: we used field data to
736 test for treatment (C, W, WL) effects on soil microbial biomass carbon and soil DOC using LME
737 models including region as a fixed effect and block as a random intercept term. We tested for
738 treatment effects on ecosystem respiration using an LME that included region as a fixed effect and
739 sampling date nested in block as a random intercept term. We tested for treatment effects on soil
740 microbial growth and respiration (per unit of soil and per unit of microbial biomass) and carbon use
741 efficiency in the western Alps experiment using LMEs including block as a random intercept term.
742 Statistical test outputs for these variables are provided in Table S1. We also used Pearson Product
743 Moment correlations to test for associations between soil microbial biomass and soil carbon
744 content, microbial respiration and growth in the western Alps experiment, irrespective of
745 experimental treatment.
746
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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. TWNW, KG and TG undertook the field work. TWNW, KG, TG, PM, CS, AB and JMA undertook the glasshouse experiment. TWNW, 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. TWNW did the data analysis, with support from DR, TWC and JMA. TWNW wrote the manuscript in close collaboration with JMA, and with support from all co-authors.

Competing interests

The authors declare no competing financial interests.

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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 content. (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 (% initial soil carbon content remaining) and cover (cm2) of each lowland plant species in the (c) western Alps (N = 36; LME: LR = 0.62, *P* = 0.4302) and (d) central Alps (N = 80; LME: 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 content. Relationship between soil temperature (ºC) 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; LR = 17.65, *P* < 0.0001), lowland plants (L; LR = 11.77, *P* = 0.0006) and their interaction (W × L; 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; µg C g^{-1}- 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 × PC# interactions (N = 20; Main Text), with different colours in (b) indicating a significant treatment × PC2 interaction. (c,d) Loadings of plant physiological measurements on (c) PC1 and

944 (d) PC2 axes. Total, belowground and aboveground biomass are sums of all plants per pot and specific leaf area (SLA),
945 maximum photosynthetic capacity (A_{max}) and stomatal conductance (g_s) are pot-wise community weighted mean (CWM)
946 values.
947
948 **Fig. S5 | Soil inoculation effects on alpine soil community composition.** Effects of low elevation soil biota
949 inoculation on (a) bacterial and (b) fungal community composition in alpine plots (N = 10 in both cases). Axes show the
950 first two components of principal coordinates analyses (PCoA; Bray Curtis distance) performed on the relative
951 abundances of bacterial and fungal operational taxonomic units, respectively. *P*-values are from PERMANOVAs testing
952 for inoculation effects on the same distance matrices.

953 ## Supplementary Tables

954 **Table S1. Linear mixed effects model outputs for effects of field treatment (control, warming, warming plus**

955 **lowland plants), region and their interaction on soil variables. LR: likelihood ratio.**

956

Response	Treatment		Region		Treatment × Region		
	LR	*P*	LR	*P*	LR	*P*	N
Soil carbon content	23.16	< 0.0001	34.53	< 0.0001	4.64	0.0984	58
Net ecosystem CO_2 exchange	23.26	< 0.0001	40.76	< 0.0001	6.43	0.0401	266
Gross primary production	58.99	< 0.0001	50.64	< 0.0001	2.78	0.2491	264
Ecosystem respiration	49.61	< 0.0001	13.08	0.0045	12.98	0.0015	268
Microbial biomass C	33.52	< 0.0001	13.64	0.0034	5.35	0.0688	58
Microbial growth (per gram soil)	9.33	0.0094	-	-	-	-	27
Microbial respiration (per gram soil)	1.20	0.5500	-	-	-	-	28
Microbial growth (biomass-specific)	8.32	0.0156	-	-	-	-	27
Microbial Respiration (biomass-specific)	6.54	0.0381	-	-	-	-	28
Microbial carbon use efficiency	15.68	0.0004	-	-	-	-	58

957

958 **Table S2. Linear mixed effects model outputs for treatment (alpine plants, lowland plants) effects on plant traits**

959 **and soil variables in the glasshouse experiment. LR: likelihood ratio.**

960

Response variable	LR	N	*P*
Pot-level plant traits			
Aboveground biomass	4.85	19	0.0277
Belowground biomass	16.31	19	0.0001
Root-to-shoot ratio	7.92	19	0.0049
Specific leaf area (SLA)	0.03	18	0.8605
Maximal photosynthetic capacity (A_{max})	4.98	19	0.0257
Maximal stomatal conductance (g_s)	14.37	20	0.0002
Soil variables			
Soil pore water absorbance (a_{350})	24.84	19	< 0.0001
Soil pore water total fluorescence (F_{tot})	27.72	19	< 0.0001
Soil pore water fluorescence index (FI)	9.55	19	0.0020
Microbial biomass carbon	0.16	19	0.6932
Fast-decaying soil carbon pool size	3.99	16	0.0458
Fast-decaying soil carbon pool decay rate	0.23	16	0.6306
Soil DOM C1 (protein-like)	21.39	19	< 0.0001
Soil DOM C2 (protein-like)	10.42	19	0.0012
Soil DOM C3 (humic-like)	15.65	19	0.0001
Soil DOM C4 (humic-like)	9.60	19	0.0019
Soil DOM C5 (humic-like)	17.39	19	< 0.0001
Soil DOM C6 (fulvic acid-like)	16.50	19	< 0.0001

961

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

963

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

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

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

966

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+

967 * Ref 57; † Ref 56