



Beyond the Tree-Line: The C₃-C₄ “Grass-Line” Can Track Global Change in the World’s Grassy Mountain Systems

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von Humboldt’s tree-line concept has dominated mountain ecology for almost two hundred years, and is considered a key indicator for monitoring change in biome boundaries and biodiversity shifts under climate change. Even though the concept of life zones and elevation gradients are a globally observed phenomenon, they have not been thoroughly explored for many contexts. One such example is the tree-line ecotone, a widely used conceptual tool to track climate change in many regions, which has limited application in the widespread tree-sparse, grassy systems that comprise a third of the world’s mountain systems. Among grasses (Poaceae), temperature is linked to variation in photosynthetic performance and community dominance for C₃ and C₄ metabolic groups, due to its role in limiting photorespiration in the C₃ photosynthesis process. Here, we investigate this community shift in grassland-dominated mountains to demonstrate the role of climate in driving this transition and discuss the potential applications of this tool to mountain ecosystem conservation worldwide. For identifying grass-dominated mountains worldwide, we measured the grass-cover using satellite data. We then compiled Poaceae distribution data for ten grass-dominated mountains spanning from 42°S to 41°N and determined the temperature intervals and elevation ranges at which each genus was found, testing for effects of temperature, precipitation, and latitudinal gradients on the dominance of C₃-C₄ grasses. Temperature was the main driver of C₃ dominance, with the richness of C₃ genera tending to surpass the taxonomic dominance of C₄ plants along mountain temperature gradients where the annual mean temperature was colder than ca. 14.6°C. Similar patterns were observed in eight out of ten mountains, suggesting that this may constitute an isotherm-driven ecotone. Consequently, this C₃-C₄ transition offers a promising tool for monitoring climate change impacts in grassy mountains. C₃-C₄ grass community shifts in response to environmental change will likely have major implications for fire frequency and severity,

rangeland productivity and livelihoods, food security, and water budgets in mountain systems. Given the severity of the implications of global change on these social-ecological systems, we propose that a "grass-line" monitoring protocol be developed for global application.

Keywords: taxonomic diversity, photosynthesis, mountain ecology, environmental gradients, climate change, community shift, Poaceae (=Gramineae)

INTRODUCTION

Mountains and Arctic systems are experiencing climate change faster than any other natural systems (Dirnböck et al., 2011; Steinbauer et al., 2018; Hock et al., 2019). In mountain systems characterized by woody natural vegetation, the tree-line is one of the primary indicators that ecologists use to measure the ecosystem-level impacts of rising temperatures, with a similar approach being followed at high boreal latitudes viz. the forest-tundra timberline (Körner, 2012; Paulsen and Körner, 2014). One of the most valuable applications of the tree-line as a tool to track climate change is that—whenever the tree-line is physically present (whether as a broad ecotone or a sharper transitional zone)—it provides a clear visual reference which can be globally assessed (Körner, 2012) and tracked over time (Paulsen and Körner, 2014). Even though this tree-line-based isotherm tracking has considerable merit in regions where there is a physical tree-line, it has limited practical applicability in regions that are naturally tree-sparse and dominated by grasslands, and which thus demand more locally suited methods and approaches (Veldman et al., 2015; Bond, 2016) in order to be equally understood under scenarios of past and future climatic change.

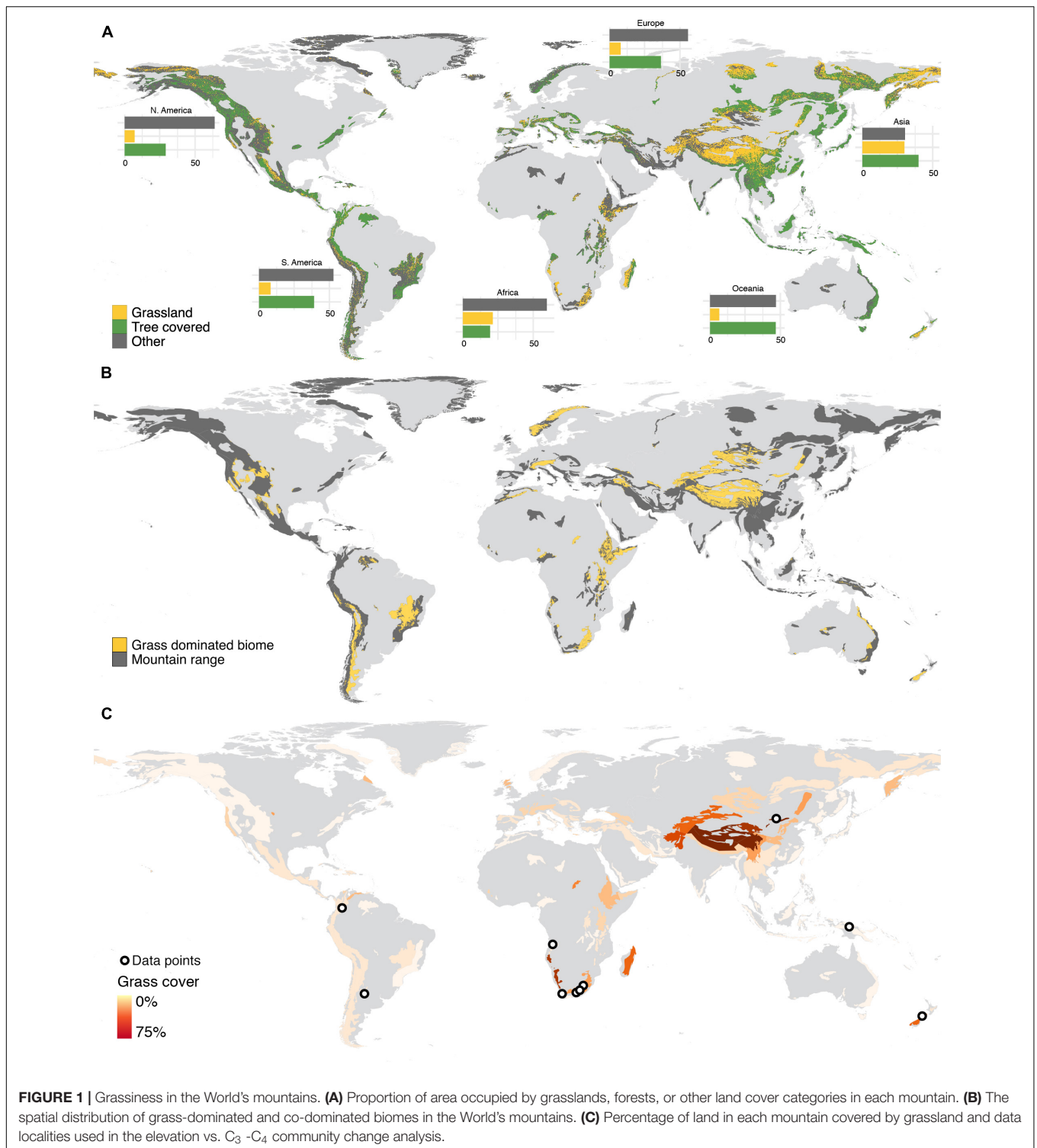
Tree-line studies have historically been centered in temperate regions of the northern hemisphere, which—for the most part—has woody vegetation (Woodward et al., 2004). The tropical areas of the Americas, Africa, and Asia also have a clear transition between woody and non-woody vegetation with elevation (Woodward et al., 2004; Körner, 2012). However, many sub-tropical and temperate systems in the southern hemisphere do not show the same pattern and are dominated by natural grassy systems, in contrast to their northern temperate and tropical equivalents (Woodward et al., 2004; Edwards and Still, 2008; Sylvester et al., 2017; Carbutt, 2019). As a result, the tree-line concept has limited to no practical value in monitoring global change in these systems.

After forests (34%), grasslands (18%) are the dominant land-cover in the World's mountains, with almost a third (31.6%) of the World's mountains having a larger proportion of grassland cover than forest cover (**Figure 1**). Grassiness is therefore an ecologically fundamental component of mountain systems globally, with grass-dominated (e.g., grasslands, meadows) or co-dominated biomes (e.g., savannas, steppes) occurring in mountain regions of all continents (except Antarctica; **Figure 1**). Although the World's most grassy mountains occur in Central Asia, southern Africa, southern South America, and New Zealand, almost all mountains worldwide have some element of grassiness (**Figure 1**). Grassy mountains provide valuable ecosystem services (Parr et al., 2014); and entire cultures,

livelihoods, and various forms of indigenous food security strategies have evolved in and around such grassy mountain systems (Maurer et al., 2006; Lavorel et al., 2011). Such montane grasslands are also often paleo-grasslands, with high floristic endemism and richness (Van Wyk and Smith, 2001; Mucina and Rutherford, 2006; Clark et al., 2011; Parr et al., 2014), while natural montane forest in these grassy mountains may be confined to lowest elevations in topographically suitable locations and fire refugia (Mucina and Rutherford, 2006; Adie et al., 2017).

In the absence of a woody component, developing alternative tools to track how communities in grassy mountains are being transformed by anthropogenic climatic change is necessary to ensure specific grassland-focused conservation strategies. Changes in physiological characteristics and/or the functional composition of grass communities can provide valuable insights on how changes in environmental gradients (like temperature) affect grass species ranges in mountains. One such relationship is the well-known differential niche occupancy of C₃ and C₄ grasses along temperature gradients. Indeed, empirical data shows that the relative taxonomic richness of C₃ grasses gradually increases with decreasing temperature found along elevation gradients (Tieszen et al., 1979; Bremond et al., 2012). This phenomenon is a result of a narrow niche overlap of the temperature tolerances in the abiotic component of the realized niches between these two photosynthetic groups, mainly due to the differences in temperature response of instantaneous net CO₂ assimilation rate and the regeneration potential of ribulose biphosphate (RuBP) and ribulose 1·5-biphosphate carboxylase/oxygenase (Rubisco) between these groups (Sage and Kubien, 2007). Available data suggests that, under enriched CO₂ environments, the Rubisco regeneration limits the distribution of C₃ plants, while CO₂ assimilation rates is restrained by temperatures lower than 20°C in C₄. While this limitation seems to be a key driver of C₃ habitat suitability, occasional acclimation to cooler environments in C₄ is empirically observed, through positive regulation of Rubisco and C₄ cycle enzymes (Sage and Kubien, 2007). However, due to its close relation to a specific temperature threshold in C₃, it is possible to identify the temperatures where most C₃ grasses have their lower elevational limits of their distributions, and to track changes in the community transition to C₄ along temperature gradients. This concept, proposed here as the "grass-line," can be tested and implemented in grassy mountain regions and provides a valuable tool to monitor temperature changes in grass dominated mountain regions where this transition occurs.

The aim of this study was, therefore, to test for a consistent/general relationship between environmental conditions and the elevation where colder temperatures allow C₃ grasses to dominate over C₄ genera. Specifically, we aim to test



if the temperature where Poaceae C_3 genera richness becomes higher than C_4 genera richness along elevation gradients is driven by similar temperature thresholds across grassland-dominated mountains, and thus, linked to an isotherm and to demonstrate how it can be applied to track temperature change in mountains missing a tree-line. By gathering distribution data for C_3 and C_4

genera of grasses on different grass-dominated or co-dominated mountains worldwide, we demonstrate the potential of using the isotherm thresholds of this ecological shift in different grassy mountains to keep track of isotherms over time and provide a basic framework to the development of a specific tracking protocol. Grassland-dominated mountains from southern

Africa, Asia, Australia, and the Americas consistently show a transition from C_4 to C_3 plants along elevation. This relationship between decreasing temperature and the C_3 - C_4 ratio along elevation indicates an unambiguous link between community photosynthetic strategy turnover and the elevational distribution of isotherms. For grassy mountains this is as ecologically significant as the tree-line, and consequently we propose the C_3 - C_4 dominance turnover transition as a "grass-line": a consistent ecological phenomenon conceptually comparable to the tree-line in that it can easily be used to monitor the ecological impacts of short- or long-term climate change using, for example, satellite imagery (Shoko et al., 2016), carbon isotopes (Collatz et al., 1998), and pollen record (Ehleringer et al., 1997; Collatz et al., 1998).

MATERIALS AND METHODS

Grass Dominated Mountains Identification and Site Selection

To identify which mountains are grassland-dominated and have low tree cover, we combined two strategies. First, we used the Global Mountain Delimitation shapefile (Rahbek et al., 2019b) and the Global Land Cover-SHARE (GLC-SHARE) databases developed by the Food and Agriculture Organization of the United Nations (FAO) (Latham et al., 2014). We summarized the land cover classification for each mountain, and then calculated the ratio of grasslands and forests cover using QGIS version 2.8.4 and R package "raster" version 3.5-2 (Hijmans, 2021). We then identified which grassland biomes were located within mountain ranges by overlaying the global mountain delimitation shapefile with the WWF's World Grassland Types (Dixon et al., 2014). We followed Dixon et al. (2014) definition of grassland biomes: "a non-wetland type with at least 10% vegetation cover, dominated or co-dominated by graminoid and forb growth forms, and where

the trees form a single-layer canopy with either less than 10% cover and 5 m height (temperate) or less than 40% cover and 8 m height (tropical)."

Biodiversity Dataset Compilation and Preparing

For sampling the occurrence data, we used both published literature surveys and the Global Biodiversity Information Facility (GBIF), as listed in **Table 1**. We selected localities comprising 40°S–41°N to represent the latitudinal range of sub-tropical to tropical mountain grasslands: the Andes (Mount Córdoba and Transecto Sumapaz, in Cordillera Oriental), the Mongolian Mountains in China, the New Guinea mountains, and southern Africa's Eastern Great Escarpment (Cape Midlands Escarpment, Mpumalanga Limpopo, Maloti-Drakensberg, Manica Highlands) and Angola's Western Escarpment. We extracted individual geographic polygons for each mountain and used the contour of the elevation features to isolate individual mountains that were generalized within larger mountain groups in Rahbek et al. (2019b). The resulting spatial polygons used for the extant analysis are available as **Supplementary Material 1**. For GBIF data, we configured the occurrence record search to include all Poaceae within each of the grassland-dominated mountains identified in the previous step, and restricted search parameters to include only preserved specimen records with geographic coordinates. We checked spelling and authorship for all genera using the collaborative database Taxonomic Name Resolution Service v4.0¹, provided by IPLant. Once we obtained a set of records for each mountain, we removed putative geographic outliers and imprecise coordinates using the R package "CoordinateCleaner" (Zizka et al., 2019). Since the intervals of occurrence were calculated without taking into account the year of sampling, it is possible that the distributions

¹<https://tnrs.biendata.org/>

TABLE 1 | Mountain grasslands and the countries in which they occur, coordinates, number of C_3 and C_4 genera, climatic type (Köppen-Geiger), and the literature source for the species lists and occurrences.

Locality	Mountain	Coordinates	C_3 genera	C_4 genera	Climatic type (Köppen-Geiger)	Source
Angola	Angolan Western Escarpment	11°30'S, 15°00'E	2	43	Sub-tropical highland variety (Cwb)	GBIF (10.15468/dl.rvu7vl)
Argentina	Mount Córdoba	32°30'S, 65°00'W	18	33	Humid sub-tropical (Cwa)	Cabido et al. (1997)
China	Mongolian Mountains	41°00'N, 110°00'E	18	11	Cold semi-arid (Bsk)	GBIF (10.15468/dl.l4sr6i)
Colombia	Cordillera Oriental	04°00'N, 74°30'W	52	41	Tropical wet and dry (As)	Giraldo-Canas (2010)
Lesotho, South Africa	Maloti-Drakensberg	29°00'S, 28°00'E	32	49	Sub-tropical highland variety (Cwb)	GBIF (10.15468/dl.nz4g3n)
Mozambique, Zimbabwe	Manica Highlands	32°30'S, 19°00'E	22	62	Humid sub-tropical (Cwa)	<i>Flora Zambeziaca</i> , Harare Herbarium (SRGH)
New Guinea	New Guinea Mountains	04°00'S, 141°00'E	55	62	Temperate oceanic (Cfb)	GBIF (10.15468/dl.l2hfex)
New Zealand	New Zealand Mountains	42°00'S, 172°00'E	45	14	Temperate oceanic (Cfb)	GBIF (10.15468/dl.hfvhiz)
South Africa	Cape Midlands Escarpment	32°00'S, 25°00'E	27	33	Cold semi-arid (Bsk)	Schonland Herbarium (GRA)
South Africa, Mozambique, Eswatini	Mpumalanga Limpopo	31°00'S, 26°30'E	19	66	Sub-tropical highland variety (Cwb)	PRECIS, Buffelskloof Nature Reserve Herbarium (BNRH)

of some genera were overestimated. Similar studies in regions with more abundant and refined records should ideally account for the year of the collection, since the temperature increased considerably in some regions during the past decades. The complete record dataset used is available in **Supplementary Material 2**. We determined the photosynthetic pathway for each genus using literature sources (Osborne et al., 2014; Fish et al., 2015), and from this calculated the proportional occurrence of C₃ and C₄ genera for each elevational interval for each mountain (**Supplementary Material 3**).

Grass Dominated Mountains in Africa

Of the 18 major mountain regions delimited for Africa by Rahbek et al. (2019b), nine have greater areas of grasslands than forests: (1) Atlas Mountains, (2) Dafur Highlands, (3) East African Highlands, (4) Ethiopian Highlands, (5) Jos Plateau, (6) Namibian/Angolan Escarpments, (7) North Somali Mountains, (8) Southern Cape region, and (9) Madagascar Highlands. African grassy mountains extend from 35°S to 35°N, and are found within the steppe, savanna, grassland, bushveld, forest, and woodland biomes.

Grass Dominated Mountains in South America

In South America, grass-dominated mountain regions (i.e., mountains with greater areas covered by grasses than forests) were found in Patagonia, Central Western Andes and the Brazilian Central Plateau. We identified eight mountains harboring grassy biomes (**Figure 1**): (1) Brazilian Central Plateau (Cerrado) and (2) Atlantic Mountains (Campos Rupestres); (3) Bolivian Huanchaca Plateau, (4) Southern Andes and Western Central Andes (Punas and Andean steppe), (5) Northern Andes (Páramos), (6) Peruvian-Bolivian Yungas (Puna, Espinal and Andean steppe), (7) Patagonia (Patagonian steppe) and (8) Venezuelan-Brazilian Tepuis (Llanos, Campinarama, Guianan savanna, and Pantepui). In these mountains, the dominant vegetation types included steppes, grasslands, shrublands, and savannas. The Cerrado, for example, has a savanna-like vegetation, harboring grasslands, shrublands and forests. There are 470 native species of Poaceae listed for the Cerrado (Filgueiras, 2021), and the most common C₄ genera are *Paspalum*, *Panicum* (C₄ and C₃), *Axonopus*, *Mesosetum*, *Aristida* (C₄ and C₃), *Andropogon*, *Eragrostis*, *Digitaria*, and *Sporobolus*. Native C₃ genera, like *Ichnanthus*, *Echinolaena*, are rare (Filgueiras, 2021).

Grass Dominated Mountains in Asia

In Asia, grass-covered mountains are found in the west (the Sinai, the Iranian Mountains, the South Western Arabian Mountains, and the Ural Mountains); in Central Asia (the Tibetan Mountains, the Pamir-Tian-Shan Highlands, the Hindu-Kush Highlands); in the North (Siberian Mountains and the Kamchatka), and in Eastern continental Asia (Qilian Mountains, Shanxi Mountains, and the Inner Mongolian Mountains), predominantly in the form of steppes, but also as bushlands and meadows. In insular Asia, grasslands are found in the alpine regions of Borneo and New Guinea, but forest cover is dominant in both mountains. In continental Asia, due to the temperate

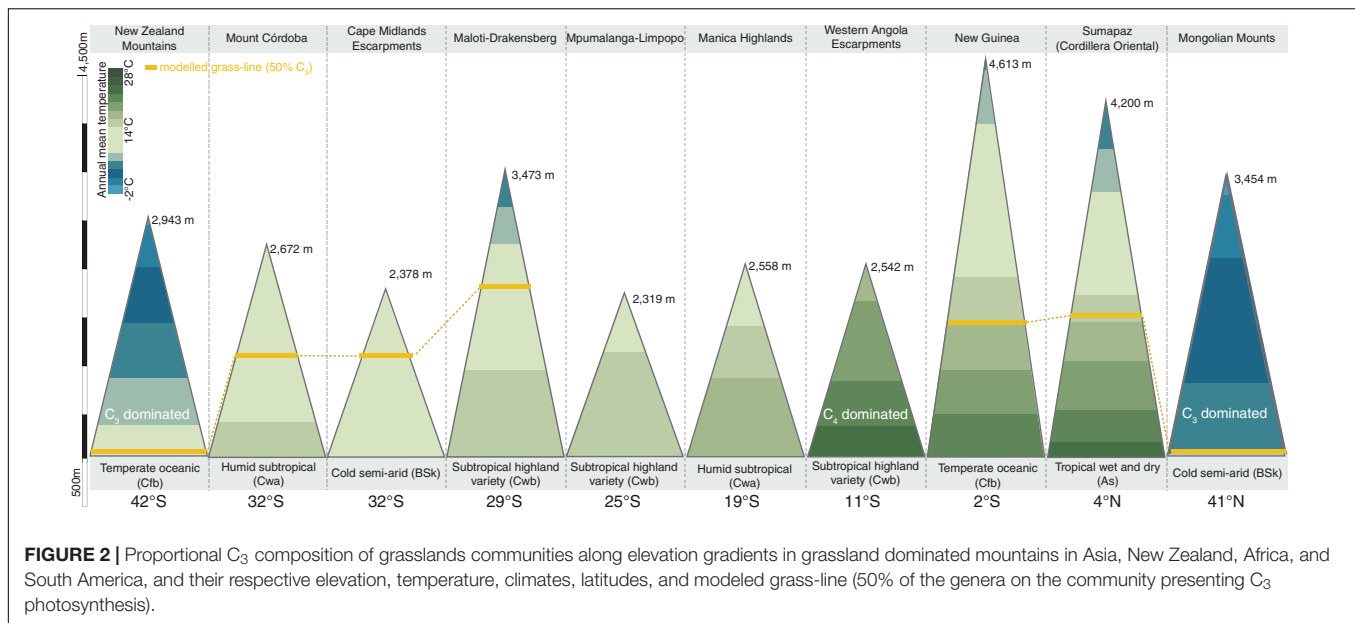
environmental conditions found in most mountains, C₃ grasses tend to dominate.

Grass Dominated Mountains in Australia/New Zealand

In Australia and New Zealand, grassland-dominated mountains are concentrated in New Zealand and in the central-western mountain regions of Australia: Flinders Range, Macdonnell Ranges, and Pilbara-Hamersley Ranges. In the Flinders Range, the southern parts of the mountains are occupied by Mount Lofty woodlands, and the western portions of Macdonnell Ranges are occupied by the Great Sandy-Tanami desert, both including grasslands as vegetation types. For Australia, the GBIF database most-recorded Poaceae genera include three C₃ genera, namely *Austrostipa*, *Rytidosperma*, and *Poa*; one C₄ genera (*Eragrostis*); and one genera with both mechanisms, *Aristida*. Meanwhile, in New Zealand, the five most sampled Poaceae genera are all C₃: *Poa*, *Rytidosperma*, *Chionochloa*, *Agrostis*, and *Festuca*.

Spatial Analysis and Statistical Analyses

We extracted the elevation data for each record using the ASTER Digital Elevation Model (30 m resolution) and then identified the elevation range (minimum and maximum values) occupied for each genus in each mountain to create presence-absence matrices using intervals of 50 meters of elevation, assuming that each genus has the potential to occupy all the elevation bands within this interval (**Supplementary Material 4**). We used WorldClim (Fick and Hijmans, 2017) bioclimatic variables (annual mean temperature, maximum temperature on the warmest month, minimum temperature in the coldest month, annual precipitation, minimum precipitation on the driest month, maximum precipitation on the wettest month) to calculate the environmental space for each elevation interval of each mountain. For this, we converted the raster layers into spatial points for each set of mountains and then reclassified them according to the same elevation intervals used in the previous step, and then calculated mean values for each variable. The WorldClim 2.0 bioclimatic variables are based on multiple weather stations' data from over 25 years between 1970 and 2000, interpolated and spatially adjusted with geographic covariates. Annual mean temperature (bio 1) is calculated as the annual mean temperature for each station during the timeframe and their mean value over the years. Maximum and minimum temperature on the warmest and coldest month (bio 5 and 6, respectively) applies the same mean temperatures dataset to identify annual extremes and average them over this timeframe. It is important to note, however, that annual mean temperature might be a biased feature at high elevations because of the great diurnal and seasonal temperature variation in mountains. Thus, this predictor requires careful interpretation, with maximum annual temperature being more biologically meaningful when discussing climatic pressures on plants' survival and development because it is a more direct proxy to the growing season, along with precipitation. The information about elevation, temperature, precipitation, and the proportion of C₃ in each elevation



range was then summarized in a single dataset, which we used to generate the plots and to produce generalized linear mixed models (GLMMs).

At the global scale, data were analyzed using GLMMs implemented using the `glm()` function, available in the base package “stats” (version 3.6.2) in the base R (R Core Team, 2020). We modeled the proportion of C₃ genera in relation to various predictors in models that incorporated a binomial distribution (with events/trials structure) and logit link function. Both slopes and intercepts were estimated for the relations between the proportion of C₃ genera and various continuous environmental variables in each mountain region. In order to minimize the effect of outlier sites where regional factors could create noise on the performance of GLMMs, mountains with *p*-values for individual models higher than 0.05 were removed from the global analysis steps. We eliminated variables that showed excessive collinearity (VIF > 5) and then used AIC criteria to select among plausible competing models. The sensitivity and specificity of each predicted threshold for annual mean temperature, maximum temperature in the warmest month and annual precipitation were calculated using confusion matrices created with the grass-line (i.e., the proportion of C₃ grasses passes 50%) indicated by the GLMMs and the number of true/false positives/negatives (i.e., C₃ > 0.5 sites) and the empirical dataset. All model fitting and sensitivity calculations are available as the **Supplementary Material 6**.

RESULTS

All continents except Antarctica host mountain-associated grass cover or grass-dominated biomes, but only in African mountains does grass cover occupy a larger area than forests (**Figures 1A,B**). Proportionally, individual mountain regions

dominated by grasslands (>50% of the area covered by grasses) were concentrated in Southern Africa, Central Asia, and New Zealand (**Figure 1C**).

We identified a consistent relationship between the temperatures found at the elevation intervals at which C₃ grasses become more representative than C₄ genera. By gathering distribution data for 245 genera of grasses (122 C₃ and 123 C₄) on 10 different grass-dominated or co-dominated mountains (**Table 1**), spanning 42°S to 41°N, we show how the temperature thresholds of this ecological shift are related to steppe-like climatic conditions found along elevational and latitudinal gradients. There is a clear latitudinal gradient in the C₃-C₄ threshold elevation (i.e., the elevation at which dominance switches from C₃ to C₄) (**Figure 2** and **Supplementary Material 7**). As expected, C₃ genera were typically associated with colder temperature conditions, becoming more dominant as temperatures decrease along the elevation gradient (**Figure 3**). Since there was a weak effect of annual mean temperature (*p* > 0.05) on C₃ proportion in Manica Highlands and Western Angola Escarpments, it was not possible to fit grass-line values for these mountains (**Table 2**). Single-factor models for annual mean temperature, the maximum temperature in the warmest month, and annual mean precipitation returned *p*-values lower than 0.05 (**Table 3**). Individual mountains models had grass-line temperature thresholds ranging from 8.94 to 17.75°C (12.46 ± 2.26°C, mean ± 95% CI, *N* = 8), and the global model estimated the grass-line as 14.6°C. The higher sensitivity and specificity values for individual predictors were 0.99 and 0.73, both for annual mean temperature. Annual precipitation specificity had the lowest ability to predict C₃ or C₄-dominated sites, with sensitivity and specificity of 0.5 and 0.47, respectively. Multiple-factor models recovered significant coefficients (*p* < 0.05) for annual mean temperature and annual precipitation (**Supplementary Material 6**). Annual mean temperature showed a higher magnitude of changes in the C₃



proportion and a negative coefficient (-0.1061), while the lowest coefficients were identified for elevation (-0.0004) and annual precipitation (0.0003). The compositional shift between C₃-C₄ in the communities (that is, >50% of the recorded genera showing C₃ photosynthesis) was identified by the multiple-factor models in sites where the mean annual temperature gets lower than 14.6°C (Figure 4). Other predictors showed more variable effects.

C₃-C₄ Turnover in African Mountain Grasslands

In the African subtropical region, C₃ genera represent a considerable proportion of the taxonomic diversity in the southernmost mountains (32°S to 28°S), with at least 27 genera found in the Cape Midlands Escarpments (vs. 33 C₄) and 32 in the Maloti-Drakensberg (vs. 49 C₄). In the Cape Midlands Escarpments, C₃ genera become more abundant than C₄ genera above 1600–1700 m, which is the upper limit of the distribution of lowland C₄ genera like *Alloteropsis*, *Hemarthria*, and *Setaria*. Above 2200 meters, the only C₄ genera found are *Cymbopogon*, *Tetrachne*, and *Pennisetum*, while C₃ genera like *Helictotrichon* and *Festuca* reach elevations up to 2400 meters. In this C₃-C₄ shift region between 1600–1700 m, mean values for annual mean temperature was 12.9°C and for annual precipitation, 749 mm. In the Maloti-Drakensberg, the elevation identified

for the community taxonomic transition between C₃ and C₄ genera was 2650 m, where annual mean temperature is ca. 9°C . C₃ genera like *Koordersiochloa*, *Stipa*, *Styppeiochloa*, and *Lamarckia* were only recorded above 2700 m, while many lowland abundant C₄ genera (*Brachiaria*, *Cynodon*, *Digitaria*, *Eleusine*, *Enneapogon*, *Hyparrhenia*, *Microchloa*, *Pennisetum*, *Setaria*, *Trachypogon*, *Tragus*) reach their upper elevational limit around 2850 m a.s.l.

Between 28°S and 10°S , the richness of C₃ genera decreases with the increase in temperature along the latitudinal gradient, while the number of C₄ genera increases. In the Mpumalanga-Limpopo mountains (26.7 to 24.7°S), for instance, we found 69 C₄ genera and 20 C₃ genera. Further north, between 20°S and 17.7°S , we listed 62 C₄ genera and 18 C₃ genera for Manica Highlands. For Mpumalanga-Limpopo, while the compositional shift gradually occurs as the temperature decreases along the elevation gradient, the grass-line is higher than the maximum elevation of the mountains, meaning that the proportion of C₃ genera does not surpass the proportion of C₄ at any point. The highest peak of Mpumalanga-Limpopo is 2319 m a.s.l., where annual mean temperature is 12.3°C and minimum annual temperature is 5°C . Around 33% of the Poaceae genera occurring at this elevation are C₃, which become gradually more common above 1500 m a.s.l., where genera like *Poa*, *Bromus*, and *Festuca* have their lower elevational boundaries. Meanwhile, several C₄ genera occur along the entire temperature gradient, like *Bothriochloa*, *Dactyloctenium*, *Paspalum*, *Schizachyrium*, *Aristida*, *Cenchrus*, *Trachypogon*, and *Eleusine*. On the other hand, Manica Highlands and Western Angola Escarpments are dominated by C₄ grasses (62 genera in Manica Highlands and 44 genera in Angola). Only two C₃ genera are recorded in Western Angola Escarpments, mostly at colder temperature sites found in high elevation belts (above 2050 m a.s.l.): *Isachne* and *Sacciolepis*. Even above 2050 m, the annual mean temperature ranges from 23.3 to 15.3°C , the minimum annual temperature ranges from 4.6 to 13.6°C , and the average annual precipitation is 1115 mm. For Manica Highlands, annual mean temperatures range from 12.9 to 17.84°C , and the average annual precipitation is 1354 mm. Since *p*-values calculated by individual models were higher than the significance threshold of 0.05 for both of these mountains, we removed them from the global model calculations and discussed their results individually.

C₃-C₄ Turnover in South America Mountain Grasslands

Grassland community gradient studies were available for Argentina (Mount Córdoba; Cabido et al., 1997) and Colombia (Transecto Sumapaz; Giraldo-Canas, 2010), and were included in our analysis. As expected, the proportion of C₃ genera increased in both areas as temperature decreases along the elevation gradient. In the Colombian Andes, the grass-line is found around 1,883 m a.s.l., where C₃ genera like *Arthrostylidium*, *Aulonemia*, *Avena*, *Calamagrostis*, *Danthonia*, *Festuca*, *Glyceria*, *Jarava*, *Nassella*, *Phalaris*, *Piptochaetium*, and *Rhipidocladum*

TABLE 2 | Individual mountains grass-line elevation and model coefficients for each generalized linear model fitted for C₃ proportion and elevation data.

Mountain	Grass-line temperature (°C)	Grass-line elevation (m)		Estimate	Std. Error	z value	Pr(> z)
Angola**	-	-	intercept	-6.743	3.587	-1.880	0.060
			coefficient	0.200	0.188	1.064	0.287
Cape Midlands	12.91	1608	intercept	4.145	1.136	3.648	0.000
			coefficient	-0.321	0.092	-3.487	0.000
Maloti-Drakensberg	8.94	2649	intercept	1.480	0.256	5.791	0.000
			coefficient	-0.166	0.023	-7.096	0.000
Manica Highlands**	-	-	intercept	-0.965	1.011	-0.954	0.340
			coefficient	-0.003	0.067	-0.043	0.966
Mpumalanga Limpopo*	10.28	2570	intercept	2.549	1.045	2.440	0.015
			coefficient	-0.248	0.070	-3.555	0.000
Mount Córdoba	11.44	1813	intercept	5.194	0.554	9.369	0.000
			coefficient	-0.454	0.042	-10.749	0.000
Sumapaz	17.75	1883	intercept	2.578	0.134	19.247	0.000
			coefficient	-0.145	0.007	-20.502	0.000
Mongolian Mounts*	9.77	839	intercept	3.176	0.859	3.698	0.000
			coefficient	-0.325	0.151	-2.154	0.031
New Guinea	16.95	2024	intercept	2.336	0.129	18.096	0.000
			coefficient	-0.138	0.007	-19.962	0.000
New Zealand*	11.62	-103	intercept	7.696	0.814	9.459	0.000
			coefficient	-0.662	0.088	-7.559	0.000

*Sites where grass-line modeled values fall beyond the current climatic conditions and elevation range of the mountain. **Sites where we recorded a weak effect of temperature or elevation on C₃ proportion ($p > 0.05$).

TABLE 3 | Single-factor models coefficients, intercepts, identified threshold for higher than 50% C₃ genera composition, sensitivity, specificity, and associated z-value probabilities.

Predictor	Model coefficient	Model intercept	Modeled threshold	Sensitivity	Specificity	Significance
Annual mean temperature (°C)	-0.1208	1.7610	14.6°C	0.9917	0.7253	$p < 0.001$
Maximum temperature in the warmest month (°C)	-0.1577	3.6735	23.3°C	0.7711	0.7059	$p < 0.001$
Annual mean precipitation (mm)	0.0001	-0.223	2589 mm	0.7397	0.6377	$p < 0.001$

have the lower elevation limits of their distribution. For this region, we calculated an annual mean temperature of 20.2°C, a minimum annual temperature of 14.7°C, and an annual precipitation of 1550 mm. For Patagonia and the Southern Andes in Argentina, four genera of C₃ grasses (*Agrostis*, *Panicum*, *Poa*, and *Vulpia*) had their lower distribution limits around 1400 m, while C₄ genera become gradually less abundant, since genera like *Andropogon*, *Axonopus*, *Gymnopogon*, and *Elionurus* have their upper elevation limit around 1550 meters. In this region, the grass-line in Mount Córdoba is found in 1813 m, where the annual mean temperature is 13°C, minimum annual temperature -7.1°C, and annual precipitation 617 mm.

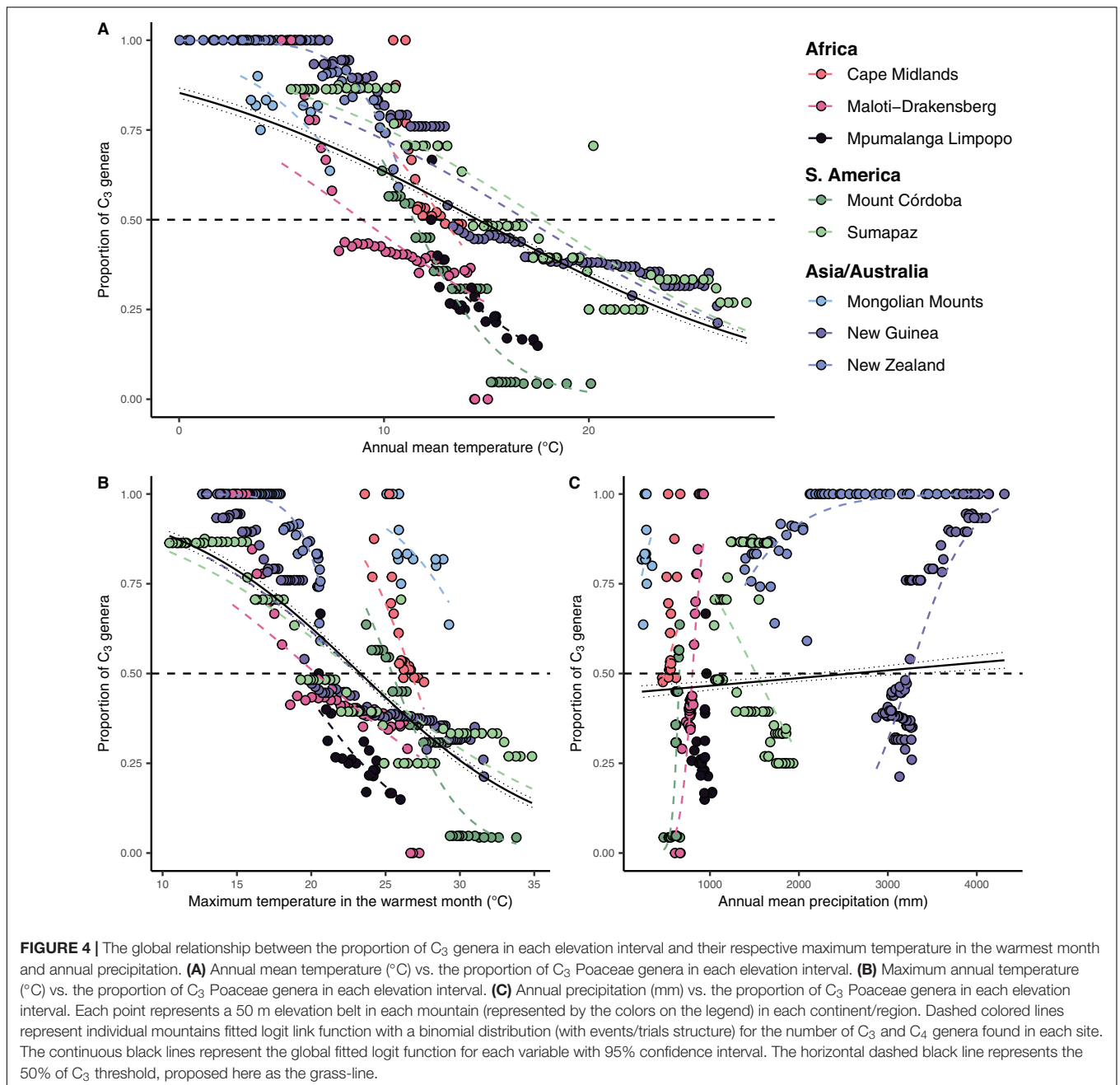
C₃-C₄ Turnover in Asian Mountain Grasslands

In the temperate Mongolian Mountains, 11 C₄ genera were found, with some occurring along the entire elevation gradient of the mountain, such as *Cenchrus*, *Cleistogenes*, and *Setaria*. There are 18 C₃ genera recorded and some are found even in low elevation sites, like *Agropyron*, *Poa*, *Elymus*, and others. Annual mean temperatures range from 3 to 7.4°C, with

maximum temperatures in the warmest month ranging from 25 to 29.3°C. In comparison, tropical mountains of Asia, like New Guinea, show a transition between C₃ and C₄ along the elevation gradient. The C₃ turnover is observed around 2050 m a.s.l., where the average annual mean temperature is around 17°C. Overall, we listed 58 C₃ genera and 64 C₄ genera for the mountains. Most C₃ genera (26 of 58) have their lower distribution limits around 2750 m (e.g., *Anthoxanthum*, *Brachypodium*, *Cyperochloa*, *Danthonia*, *Dichelachne*, *Isachne*, and *Trisetum*), and only six C₄ genera have records above 2800 m (*Arthraxon*, *Arundinella*, *Capillipedium*, *Coix*, *Digitaria*, and *Dimeria*).

C₃-C₄ Turnover in Australia and New Zealand

In the New Zealand Mountains, we listed 45 C₃ and 14 C₄ genera, and identified occurrences of C₄ genera like *Digitaria*, *Cenchrus*, *Echinochloa*, and others in warmer sites, in elevations below 1050 m, but mountains are almost entirely dominated by C₃ genera. The dominant climate in New Zealand mountains is Temperate



oceanic (Cfb), with annual mean temperatures ranging from 0 to 10.7°C.

DISCUSSION

The Transition Between C₃ and C₄ in Grassy Mountains Is Driven Mainly by Temperature

The turnover from C₄ to C₃ dominated grasslands with decreasing temperature along the elevation gradient is transitional and is not in the form of a sharply demarcated line

(Figure 3). It occurs along an isotherm, a temperature-linked elevation interval, which has a stronger effect than precipitation and elevation itself. The same phenomenon occurs with tree-lines, which are very often a transitional zone distributed over hundreds of meters. Transitional change does not mean that the grass-line cannot be used for monitoring as logistic regression models can provide temperatures and elevations associated with probabilities of turnover from C₄ to C₃ dominated grasslands. Based on our results, we argue that identifying the elevation interval where the annual mean temperature is around 14.6°C is indicative of the elevation range where C₃ genera become more representative than C₄, especially for subtropical grassy

mountains. While there is a range of variation in the grass-line temperature for empirical datasets, the average value of 14.6°C can be used as an initial reference to the development of locally suited plots focused on tracking changes in the "grass-line" over time, since the overall pattern is effectively demonstrated: lower temperature sites show higher probabilities of being dominated by C₃ genera. Here, we also present a model of the general guidelines for the development of such protocols using the Maloti-Drakensberg as an example.

Several factors like phenotypic plasticity, C₄ acclimatization, microclimatic variation, edaphic factors, biologic interactions, regional genera pool, biogeographic histories of individual mountains, and even methodological limitations (spatial resolution of the mountain delimitation polygons, climatic datasets, and digital elevation models) could influence the results observed for Angola and Manica Highlands, and affect the precision of the global model. Thus, the global model is a starting point to the development of individual mountain-focused protocols, and elevation intervals need to be locally identified and adapted to be effectively applied for the development of locally suitable plot designs to track the changes in C₃-C₄ composition over time and the point of inflection where the communities along the elevation gradient begin to become C₃ dominated. It is important to replicate our analysis in individual sites and test for further convergence of temperatures linked to the grass-line in each mountain before applying the protocol. This is necessary since our results are limited by sample size, as grass-lines are physically present in five of the ten mountains we analyzed. Even for modeled grass-lines, like the ones we identified for Mpumalanga Limpopo, Mongolian Mountains, and New Zealand, there was a considerable variation in annual mean temperature thresholds, suggesting that the grass-line may be a more suitable tool for certain localities than others (like Manica Highlands and Angola, for example). For these tropical mountains where the transition was not empirically observed, the combination of warm temperatures and a limited regional pool of C₃ genera (only 2 of 45 Poaceae genera found in Angola are C₃) justifies the low effect of annual temperature on the increase of C₃ in Angola. On the other hand, the tropical conditions and the relative lower elevation extent (2558 m) in Manica Highlands, when compared to other tropical mountains included in our analysis (4613 m in New Guinea and 4200 m in Mount Sumapaz) do not allow for the temperature gradient to reach the same temperate conditions observed for higher elevation belts of other tropical mountains. This result suggests that the grass-line is more efficient for subtropical mountains, which already have a local pool of C₃ species and where the elevation gradient reaches temperatures lower than the global threshold of 14.6°C.

This C₃-C₄ grass-line has a dynamic temporal history, with isotope reconstructions indicating that past climates greatly affected C₃-C₄ proportional dominance globally; data indicate a pronounced increase in C₄ dominance for both sub-tropical and tropical regions during cold and dry periods, like the Last Glacial Maximum (Ehleringer et al., 1997; Collatz et al., 1998; Osborne, 2008; Edwards et al., 2010). Controversially, available data suggest that even though C₃ grasses might have historically benefited

from a carbon-rich atmosphere over C₄ species (Ehleringer et al., 1997; Collatz et al., 1998; Osborne, 2008; Edwards et al., 2010), temperature is still one of the overriding determinants of their dominance (Collatz et al., 1998; Edwards and Still, 2008). Even though the evolution of C₄ grasses seems to be strongly influenced by warmer climatic conditions (Sage, 2001; Sage et al., 2018), in an evolutionary context, however, temperature change itself is not capable of explaining C₄ plant evolution (Sage, 2004). Instead, it is likely that complex interactions between biological, atmospheric, and geologic elements interacted (Sage, 2004). Interestingly, even though current CO₂ levels are rising dramatically, C₃ plants still employ substantial metabolic effort in sustaining a photosynthetic apparatus capable of handling low CO₂ concentrations (Sage and Coleman, 2001).

This pattern is a direct consequence of the relatively poor photosynthetic performance of C₃ plants under warm climatic conditions, a well known as physiological limitation caused by the lower specificity of RuBisCO under such conditions, resulting in a metabolic waste of energy in the form of photorespiration. As a result, in cooler mountains (such as New Zealand and northern China), the whole elevation continuum is dominated by C₃ genera, while warmer tropical mountains (such as the Angolan Highlands) are dominated by C₄ genera (**Figure 2**). For example, in New Zealand, while there are occurrences of C₄ genera at warmer belts found in low elevations (below 1050 m), all the upper belts are dominated by C₃ genera, while in China given the remarked temperate climate found in these mountains, most elevation belts present a relatively stable 70% C₃ composition, with no clear increase along the elevation gradient (**Supplementary Material 7**). Even in tropical mountains like the Northern Andes, where the climatic heterogeneity is considerably higher (Rahbek et al., 2019a), C₃ genera like *Alopecurus*, *Aphanelytrum*, *Brachypodium*, *Cinna*, *Cortaderia*, *Elymus*, *Neurolepis*, *Phleum*, *Ortachne*, *Triniochloa*, and *Trisetum* only become dominant above 2450 m a.s.l., where the mean annual temperatures become lower than 14.6°C. It is noteworthy though, that air temperature *per se*, despite being highly correlated, is not a direct measure of leaf temperature for low stature functional groups, which is usually warmer than the atmospheric measure because of boundary layer effects. Also, since WorldClim variables are interpolated 2 m above ground-level weather-station data, we recognize that our temperature data is possibly not refined enough to capture more local scale dynamics. In mountain systems where there is snowfall, grasses might also be periodically isolated from atmospheric conditions whenever there is snow accumulation. For trees, this is not observed, and it is one of the reasons for the sharper tree-line limit observed in forest-dominated mountains (Körner, 2003; Scherrer and Körner, 2010; Scherrer and Körner, 2011).

As the World's grassy mountain systems are most extensive in the moisture-stressed mid-latitudes, moisture availability (or moisture reliability) may favor C₃ over C₄ richness in these regions. However, precipitation itself seems to play a weak role (**Figure 4** and **Supplementary Table 3**) in this community shift at the local gradient level, even though it may play an important role in explaining variation among regions (Edwards and Still, 2008) and also the global dominance of C₄

grasses (Adie et al., 2017). Our models therefore reinforce the role of temperature as the primary driver of the C₃-C₄ "grass-line," in the same way that it determines the global distribution of C₃ and C₄ grass communities, surpassing even the effects of the latitudinal gradient.

One immediate implication is that—with temperature increases of 1.5°C projected for the next 50 years under current emission scenarios (Hock et al., 2019)—as the C₃-C₄ threshold shifts upslope under warmer temperatures, montane C₃ grasses are likely to undergo contractions in their distributions. Narrow endemic genera (like *Arthrostylidium*, *Aulonemia*, *Calotropis*, *Danthonia*, *Deyeuxia*, *Rytidosperma*, *Sacciolepis*, *Trichopteryx*, and *Vulpia*) may be at most risk from population bottlenecks and a decrease in photosynthetic productivity, or even local extinction. Meanwhile, C₃ dominated mountains (e.g., temperate mountains in New Zealand and China) will face an increase in the presence and dominance of C₄ species and higher vulnerability to invasions of alien C₄ grasses. Thus, biodiversity erosion will certainly be a consequence of increased temperatures in currently C₃-dominated mountains, due to the predicted warming of originally colder sites found in higher elevation in other sub-tropical mountains.

Developing a Grass-Line Protocol: How Can we Track the Transition in Grassy Mountains?

While developing strategies to track the grass-line is challenging and needs to be continuously developed, we outline some basics for such study designs when monitoring grassy mountains. Thus, the geographic distribution and the sizes of the plots—ideally large permanent rectangular plots with randomly distributed smaller sampling plots—need to be carefully planned and evaluated for each region.

As a case in point, we hereby propose a pilot protocol to monitor C₃-C₄ grass community shifts in grassy mountains using the Maloti-Drakensberg as an example. We propose a permanent nested-plot-based design that can be revisited every 2–5 years, with more frequent surveys during years of environmental extremes. Permanent plots should span the whole ecotone (i.e., cover an elevational gradient), centered approximately on the grass-line calculated for a given mountain. Target areas can be delimited using GIS tools to maximize the inclusion of grassland transects known to include both C₃ and C₄ genera, where multiple permanent larger rectangular plots (e.g., 100 × 50 m) are established along the elevation gradient with smaller 1 × 1 m subplots systematically sampled to measure presence/absence for indicative grass genera (Figure 5). With this data, it becomes possible to calculate the relative abundance of C₃ vs. C₄ grasses for the large plots and to monitor shifts in the distribution of indicative genera along the elevational gradient (in the Maloti-Drakensberg the transition would typically start around 1850–2000 m in elevation, reaching 50% around 2650 m). Additionally, this protocol would allow for monitoring the occurrence and abundance of native and invasive C₄ genera above the predicted elevational threshold over time, as well as monitoring population declines in indicator C₃ genera occurring in high-elevation

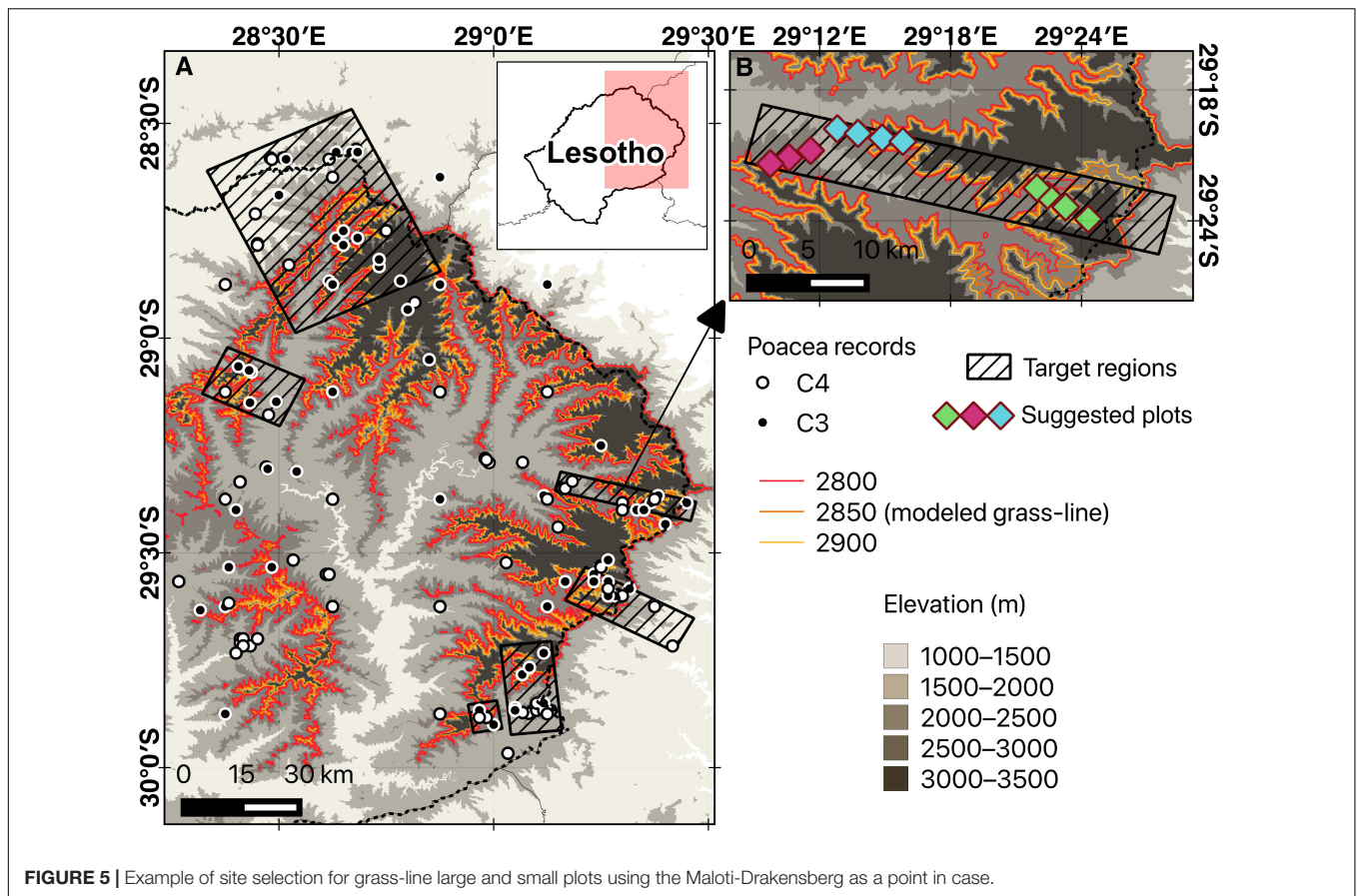
sites (e.g., above 1850 m a. s. l. in the Maloti-Drakensberg). By monitoring the "grass-line" over time, while simultaneously tracking ongoing temperature changes, it will be possible to estimate the habitat loss for C₃ genera, the rate of expansion of C₄ genera, and, as a result, the increasing vulnerability of grass-dominated mountain peaks to lowland taxa (as reported for tree-dominated temperate mountains by Steinbauer et al., 2018). Overall, the protocol needs to take into account the individual environmental characteristics of each region and the diversity of genera found in each elevation associated with the transition to be able to capture changes in this composition over time.

The grass-line is complementary to monitoring the elevational limits of individual species, but it is preferred since it provides a generalized approach. It uses a functional/physiological metric that is less affected by, for example, non-thermal distributional boundaries of native species which may be limited to specific geologies or soil moisture levels and will not respond to changes in temperature; or the establishment of alien species, which may cause big changes in species composition, but are unrelated to changes in climate. The grass-line offers the same advantage as (other) functional trait approaches—it allows us to compare areas irrespective of species composition, using a functional/physiological metric that has direct ecological relevance.

Under changing environmental conditions we expect to see some degree of idiosyncrasy in the responses of individual species, and using a "community-level" metric will provide a better indicator of how the ecosystem is changing. As in tree-line studies, "grass-line" ecotone behavior is best monitored through tracking the growth and movement of C₃ and C₄ genera/species in fixed plots, resampled at intervals. A similar protocol is the Mountain Invasion Research Network (MIREN) that determines the behavior of non-native species in mountains. By designing a protocol that can be replicated across the grass-line ecotone, and repeatedly sampled at temporal intervals we can determine the behavior of the grass-line on a large scale.

Future Climatic Scenarios Impact on This Composition Shift and the Importance of a Grass Focused Protocol

The social-ecological impacts of shifts in the C₃-C₄ "grass-line" on culture, livelihoods, and rangeland-dependent food security will be likely considerable, and will require major adaptations in both (1) fire management—given that higher temperatures favor fire-prone C₄ grasses over fire-retarding C₃ grasses (Clark et al., 2009)—and (2) grazing governance—as natural pasture palatability will change in response to grass community changes; grazing management will need to adapt accordingly to become sustainable (Clark et al., 2009). The currently available evidence is not conclusive about quantitative implications of the C₄ to C₃ shifts in the cascading ecological effects of this community transition and its net impact on herbivores (Chamaille-Jammes and Bond, 2010). Therefore, developing a protocol monitoring grassland community shifts in C₃-C₄ generic composition offers a valuable tool to quantify and to identify sites where this shift may occur. Moreover, monitoring "grass-line" boundaries can clarify



the real impacts of regional community changes in phenology, digestibility, and productivity, since they may vary substantially between C₃ and C₄ grasses, and these traits are likely to affect the diets of large mammalian herbivores (Ehleringer et al., 2002). Among these groups, productivity is also a complex function influenced by multiple environmental conditions (e.g., daytime growing season temperature, nitrogen, water, carbon), making it difficult to establish a generalizable differentiation and to predict precisely expected local changes, reinforcing the necessity to monitor and measure this shift at regional scales (Chamaillé-Jammes and Bond, 2010). There will likely be both “winners” and “losers” from such changes. For example, longer growing seasons may increase carry capacity in some regions (provided aridity does not also increase), and many C₄ grasses may be palatable than C₃ grasses (e.g., C₄ *Themeda* over C₃ *Tenaxia* in southern Africa) (Clark et al., 2009) suggesting a switch to more favorable natural pastures. All these peculiarities reinforce the need for monitoring this change in multiple scales across different environments, and the “grass-line” concept provides a suitable protocol for this purpose.

For many of the World’s mountains there is evidence that sustained increases in temperature over the past century and land-use related disturbances have promoted upward elevation shifts in the tree-line (Ameztegui et al., 2016; Cazzolla Gatti et al., 2019) as well as increasing incidence of “lowland” taxa at novel higher elevations, with standardized

global protocols being used to track these ecological responses (Grabherr et al., 2000; Naccarella et al., 2020). Historical compositional shift datasets are available to many temperature mountains (mostly in western Europe), and they provide important evidences of how climate change is affecting mountains biodiversity and how conservation efforts can be directed to mitigate these impacts (Steinbauer et al., 2018). At present, there are no comparable data for grassy mountain systems, especially in the southern hemisphere. We suggest that a common global protocol to monitor the C₃-C₄ “grass-line” in grassy mountain systems may provide equivalent data for tracking change and providing scientific support for climate adaptation programs in such mountains.

DATA AVAILABILITY STATEMENT

The datasets are available as **Supplementary Material**.

AUTHOR CONTRIBUTIONS

JD developed the methodology, collected and analyzed the data, prepared the figures, developed the protocol, and wrote the foundation manuscript and supplementary data. PR provided the data,

developed the protocol, and assisted with constructing the body text. SJ assisted with data analysis and figures, and edited the manuscript. MB assisted on the discussion of the results, developed the protocol, and edited the manuscript. VC conceived the study, collected and provided field data, assisted with constructing the body text, and provided project leadership. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2021.760118/full#supplementary-material>

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