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► To cite this version:

Sylvia Haider, Jonas J. Lembrechts, Keith Mcdougall, Anibal Pauchard, Jake M. Alexander, et al.. Think globally, measure locally: The MIREN standardized protocol for monitoring plant species distributions along elevation gradients. *Ecology and Evolution*, 2022, 12 (2), 10.1002/ece3.8590 . hal-03619105

HAL Id: hal-03619105

<https://u-picardie.hal.science/hal-03619105>

Submitted on 4 Oct 2022

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Think globally, measure locally: The MIREN standardized protocol for monitoring plant species distributions along elevation gradients

Sylvia Haider^{1,2,x}, Jonas J. Lembrechts^{3,x}, Keith McDougall⁴, Aníbal Pauchard^{5,6}, Jake M. Alexander⁷, Agustina Barros⁸, Lohengrin A. Cavieres^{9,6}, Irfan Rashid¹⁰, Lisa J. Rew¹¹, Alla Aleksanyan^{12,13}, José R. Arévalo¹⁴, Valeria Aschero⁸, Chelsea Chisholm⁷, V. Ralph Clark¹⁵, Jan Clavel³, Curtis Daehler¹⁶, Pervaiz A. Dar¹⁷, Hansjörg Dietz⁷, Romina D. Dimarco^{18,19}, Peter Edwards⁷, Franz Essl²⁰, Eduardo Fuentes-Lillo^{5,6,3,21}, Antoine Guisan²², Onalemma Gwate¹⁵, Anna Hargreaves²³, Gabi Jakobs⁷, Alejandra Jiménez^{5,6}, Paul Kardol²⁴, Christoph Kueffer^{7,25}, Christian Larson¹¹, Jonathan Lenoir²⁶, Bernd Lenzner²⁰, Miguel A. Padrón Mederos¹⁴, Maritza Mihoc^{9,6}, Ann Milbau²⁷, John W. Morgan²⁸, Jana Müllerová²⁹, Bridgett Naylor³⁰, Ivan Nijs³, Martin A. Nuñez^{19,31}, Rüdiger Otto¹⁴, Niels Preuk¹, Amanda Ratier Backes^{1,2}, Zafar A. Reshi¹⁰, Sabine B. Rumpf³², Verónica Sandoya^{33,34,35}, Mellea Schroder³⁶, Karina L. Speziale³⁷, Davnah Urbach³⁸, Graciela Valencia^{9,6}, Vigdis Vandvik³⁹, Michaela Vitková⁴⁰, Tom Vorstenbosch^{20,41}, Tom W.N. Walker^{7,42}, Neville Walsh⁴³, Genevieve Wright⁴⁴, Shengwei Zong⁴⁵, Tim Seipel¹¹

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Abstract

Climate change and other global change drivers threaten plant diversity in mountains worldwide. A widely documented response to such environmental modifications is for plant species to change their elevational ranges. Range shifts are often idiosyncratic and difficult to generalize, partly due to variation in sampling methods. There is thus a need for a standardized monitoring strategy that can be applied across mountain regions to assess distribution changes and community turnover of native and non-native plant species over space and time. Here, we present a conceptually intuitive and standardized protocol developed by the Mountain Invasion Research Network (MIREN) to systematically quantify global patterns of native and non-native species distributions along elevation gradients and shifts arising from interactive effects of climate change and human disturbance. Usually repeated every five years, surveys consist of 20 sample sites located at equal elevation increments along three replicate roads per sampling region. At each site, three plots extend from the side of a mountain road into surrounding natural vegetation. The protocol has been successfully used in 18 regions worldwide from 2007 to present. Analyses of one point in time already generated some salient results, and revealed region-specific elevational patterns of native plant species richness, but a globally consistent elevational decline in non-native species richness. Non-native plants were also more abundant directly adjacent to road edges, suggesting that disturbed roadsides serve as a vector for invasions into mountains. From the upcoming analyses of time series even more exciting results especially about range shifts can be expected. Implementing the protocol in more mountain regions globally would help to generate a more complete picture of how global change alters species distributions. This would inform conservation policy in mountain ecosystems, where some conservation policies remain poorly implemented.

Keywords: climate change, invasive species, long-term ecological monitoring, MIREN, mountain biodiversity, Mountain Invasion Research Network, range dynamics, range expansions

118 **Introduction**

119 Mountains are biodiversity hotspots and provide a wealth of ecosystem functions and benefits to people
 120 (Körner & Spehn, 2002; Martín-López et al., 2019; Mengist et al., 2020). At the same time, mountain
 121 ecosystems are particularly susceptible to global change. For instance, temperatures are increasing faster at
 122 high elevation than at low elevation (Nogués-Bravo et al., 2007; Pepin et al., 2015). In the alpine zone of
 123 the European Alps, temperatures have increased approximately twice as much as the northern hemisphere
 124 average over the past 100 years (Gobiet et al., 2014). Importantly, amplified warming has enabled many
 125 plant species to move to higher elevation (Lenoir et al., 2008; Pauli et al., 2012; Steinbauer et al., 2018).
 126 For instance, between 1971 and 1993 native plant species from the forest understorey in the French
 127 mountains shifted their elevational range uphill at an average rate of 38 m per decade (Lenoir et al. 2008).
 128 Another prominent example is the observed upward shift of most vascular taxa at Chimborazo in Ecuador
 129 since Alexander von Humboldt's visit more than two centuries ago (Morueta-Holme et al., 2015). An
 130 expected consequence of such uphill migrations of more competitive lowland species is that less
 131 competitive alpine species might locally become extinct on mountain summits (Dullinger et al., 2012;
 132 Alexander et al., 2018; Guisan et al., 2019; Rumpf et al., 2019). Such local extinctions were recently
 133 documented for birds (e.g. Freeman et al., 2018).

134 In addition to temperature increase, human activities in mountain areas have changed markedly over the
 135 last decades (e.g. Peters et al., 2019; Wang et al., 2019; for an overview see Payne et al., 2020). Mountain
 136 land use has intensified in many places across the globe (Spehn et al., 2006), driven by booming tourism
 137 industries (Pickering & Barros, 2012; Debarbieux et al., 2014), overexploitation of natural resources and
 138 ever-increasing demands for agricultural land (e.g. Gillet et al., 2016; Ross et al., 2017). The abandonment
 139 of traditional cutting and grazing practices has also occurred in some mountain regions (e.g. MacDonald et
 140 al., 2000). Both land use intensification and abandonment can alter plant species distributions and diversity
 141 alone (Pellissier et al., 2013; Alexander et al., 2016) and by interacting with climate change (Guo et al.,
 142 2018; Elsen et al., 2020).

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Further, previously remote areas are becoming increasingly accessible due to construction of new roads and trails, which not only cause a direct disturbance, but also act as corridors for plant species movements (Ansong & Pickering, 2013; Lembrechts et al., 2017; Rew et al., 2018). The role of roads as dispersal corridors is amplified due to increased vehicle traffic, often as a result of recreation and tourism (e.g. Müllerová et al., 2011). Roadside habitats also provide ideal spaces for non-native plants, which generally benefit from reduced competition, increased soil nutrients, more favourable microclimatic and hydrological conditions and intermediate disturbance (Müllerová et al., 2011; Averett et al., 2016). Thus, both native and non-native plant species are known to disperse along mountain roads, from low to high elevation and *vice versa* (Dainese et al., 2017; Lembrechts et al., 2017; Guo et al., 2018). Indeed, many high elevation areas once free of lowland and non-native species but connected to lowlands by road networks are now harbouring lowland and non-native plant species. Examples for this are the volcanoes of the Hawaiian archipelago (Jakobs et al., 2010), the high Andes (Barros et al., 2020) and the Teide National Park on Tenerife (Dickson et al., 1987). Roadside habitats are also conduits for non-native plants to spread into natural vegetation once established along roadsides (Alexander et al., 2011; Seipel et al., 2012). The elevational redistribution of plant species, especially non-native species (Dainese et al., 2017), has already significantly impacted mountain ecosystems (Guo et al., 2018) and will continue to do so in the future (Petitpierre et al., 2016). For example, non-native plants can cause biotic homogenization (Haider et al., 2018), reduce the diversity of local native species (Daehler, 2005) and affect important ecosystem functions and services (McDougall et al., 2011b; Pecl et al., 2017). In the mountains of Iceland, non-native *Lupinus nootkatensis* competes strongly with native plant species and modifies soil properties through nitrogen fixation (Wasowicz, 2016). In the alpine zone of the central Chilean Andes, non-native *Taraxacum officinale* shares pollinators with several native Asteraceae species (Muñoz & Cavieres, 2019), reducing pollinator-visitation rates and seed-set where *T. officinale* is at high abundances (Muñoz & Cavieres, 2008). Finally, uphill migration of non-native trees and shrubs can increase fire risk at high elevation (Cóbar-Carranza et al., 2014), and transform plant communities through competition (Zong et al., 2016; Nuñez et al., 2017).

While human-driven vegetation change can happen relatively quickly in mountains, it often only becomes apparent at temporal scales beyond the few years covered by most ecological experiments (Mirtl et al., 2018). Thus, data from long-term time series in mountains are essential to identify and follow changes in plant communities (Pauli et al., 2012). There are currently two main types of initiatives which monitor high-elevation vegetation change. At the local or regional scale, some well-established long-term monitoring sites follow a holistic approach and document not only floristic changes, but also modifications for example of soil, hydrology or atmospheric conditions. Examples are Niwot Ridge in the Colorado Rocky Mountains (www.nwt.lternet.edu) or the Sierra Nevada Global Change Observatory in Spain (<https://obsnev.es/en/>). At the global scale, the Global Observation Research Initiative in Alpine Environments (GLORIA, www.gloria.ac.at; Pauli et al., 2015) is a network monitoring floristic change on mountain summits with a standardized approach. What would complement these highly valuable approaches, is a global long-term monitoring network that covers the full vertical extents of different mountain regions and that allows the detection of species responses to both climate and other human activities.

Here, we present a standardized protocol for monitoring changes in the elevational distribution, abundance and composition of plant biodiversity in mountains as a result of the interaction between climate and human pressures. Importantly, the protocol focuses on large elevation gradients (>1700 m on average; ranging from c. 700 m to >4000 m), allowing vegetation change to be monitored across a broad range of climates and plant community types. It explicitly contrasts anthropogenically disturbed and (semi-)natural vegetation within sampling sites, thus increasing detection of rapid community changes and providing greater insight into the drivers of change. The protocol has been developed by the Mountain Invasion Research Network (MIREN, www.mountaininvasions.org) (Kueffer et al., 2014), a network initially founded in 2005 to study patterns and processes of non-native plant invasions in mountains and recently expanded to more widely understand the effects of global change on mountain plant biodiversity and the distribution of species. The protocol provides a conceptually intuitive yet comprehensive and standardized way to record and monitor native and non-native species along elevation gradients. The survey has been running in some mountain regions of the world since 2007 (Alexander et al., 2011; Seipel et al., 2012) and

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3 195 continues to be implemented in new regions. In this paper, we summarize the most important findings
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5 196 gained over the time of using this protocol, discuss its strengths and weaknesses and outline opportunities
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7 197 and challenges for future work. To achieve broad reach and long-term maintenance of sites, monitoring
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9 198 protocols must be simple, efficient, and inexpensive. Our intention is to promote the use of the MIREN
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11 199 road survey protocol to monitor biodiversity change in mountains, and to generate global, regional and local
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13 200 insights into how plant species and communities are responding to rapid global change in mountains.
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18 202 **Materials and Methods**
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20 203 *The Mountain Invasion Research Network*
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22 204 The Mountain Invasion Research Network (MIREN) has been founded in 2005 as a first global effort to
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24 205 apply the known principles from plant invasion ecology in mountainous environments (Kueffer et al., 2008;
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26 206 Kueffer et al., 2009). From the start, the main goal has been to link detailed observations at the local-scale
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28 207 from a broad range of mountain regions, to come to global conclusions on common patterns (and divergence
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30 208 from them) regarding mountain plant biodiversity (Kueffer et al., 2014). The core of the network has been
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32 209 the underlying road survey protocol, which allowed flexible application all across the world, yet a
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34 210 standardized baseline of data collection that could be maintained for a long time. While research topics and
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36 211 techniques have diverged throughout the years, the core business of MIREN remains to increase the spatial
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43 214 *Survey design*
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45 215 The MIREN road survey is conducted by region. In each region, participants select three sample roads that
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47 216 extend over a broad elevation gradient, ideally reaching elevations beyond the treeline (for examples, see
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49 217 Figure 1). We define a region as an area in the same biogeographical unit containing similar flora, geology
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51 218 and elevational ranges, usually with distances between roads of less than 150 km (Figure 2). Selected roads
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53 219 should begin at the bottom of the mountain region, in a valley, at sea level or where no further elevation
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55 220 change occurs, and reach the highest elevation typical for roads in the region. Roads can be gravel or paved
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but should be open for public vehicle traffic for at least some part of the year. Once roads have been selected, the elevational range of each road is divided into 19 equally wide elevational bands from the lowest to highest possible sampling location, giving a total of 20 sample sites per road located at the splits between elevational bands. Sample sites are determined prior to going into the field and located as precisely as possible using a global positioning system (GPS). At each sample site, three 2 m × 50 m plots are laid out in the form of a “T”: one plot (the top of the “T”) is parallel to the road. The other two plots extend end-to-end and perpendicular to the road, starting from the centre of the first plot, with midpoints at 25 m and 75 m from the roadside plot (Figure 3). The same plots are resurveyed every five years. If the plot locations have to be changed due to unforeseen circumstances, new sites are placed as near as possible and geolocated.

For each plot, a few basic environmental variables have to be collected in the field (e.g. tree cover; see Supporting Information S1). However, we aim to keep the protocol as simple as possible, and therefore additional variables of interest can be either extracted from online resources (e.g. soil characteristics, Hengl et al., 2017) or topographic variables (Amatulli et al., 2018) or through optional add-on studies (see section below).

Plant species surveys

Within each of the three plots at all 20 sample sites along the three roads, observers record all vascular plant species (including both native and non-native species) and visually estimate vegetation cover (eight percentage cover classes with 1=<0.1%, 2=0.1-1%, 3=2-5%, 4=6-10%, 5=11-25%, 6=26-50%, 7=51-75%, 8=76-100%) and record abundance (number of individuals in three classes; 1=1-10 individuals (or ramets), 2=11-100 individuals, 3=>100 individuals) of each species. The detailed sampling protocol is provided in Supporting Information S1 and can also be downloaded from the MIREN website (www.mountaininvasions.org). Taxa should be identified to species level using up-to-date local floras. Before being included in the global database, submitted regional species lists undergo taxonomic harmonization to detect synonyms for the same species in different regions, and to correct spelling

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247 problems. This procedure is done by the MIREN data managers, using the R-packages “taxize”
248 (Chamberlain & Szöcs, 2013; Chamberlain et al., 2020) and “WorldFlora” (Kindt, 2020). Firstly, species
249 names are matched with World Flora Online (<http://www.worldfloraonline.org>), and if not found there, they
250 are searched via the additional databases included in the Taxonomic Names Resolution Service (Boyle et
251 al., 2013). All changes of species names are transmitted to the submitting region for verification or
252 correction, before the dataset enters the global database (Figure 2).

253 Each taxon should be classified as native or non-native to that region by the participant using local floras
254 and databases. As a general rule, plant species introduced into the country or mountain range after AD 1500
255 are considered as non-native, although regional deviations are welcome if properly justified. For noteworthy
256 records (e.g. first records or new high/low elevation records of native or non-native species), specimens
257 should be collected outside of the plots (when possible) and placed in a herbarium to facilitate identification
258 and to inform local floras (Walsh & McDougall, 2018).



Figure 1: Examples of roads in the landscape (a-c) and key non-native species (d-f) across a range of MIREN regions. (a) Harsh mountain climates (here the Cañadas del Teide on Tenerife (Canary Islands, Spain) have traditionally been seen as an adequate barrier against non-native plant invasion; (b) the direct local impact of roadside disturbance on mountain plants is visible on native *Azorella* cushion plants along a road in the dry Andes near Mendoza, Argentina; (c) interactive effects of climate and land use, exemplified by dramatic differences in snow cover on versus beside a mountain road in northern Norway; (d) *Taraxacum officinale*, one of the most widespread non-native plant species along MIREN mountain roads (Seipel et al., 2012), in a sample plot on a volcanic gravel slope in the Argentine Andes; (e) non-native *Verbascum thapsus* on a roadside in the highly invaded lowlands of the Andes in central Chile; (f) *Trifolium pratense* in northern Norway, where the species is rapidly moving uphill along mountain roadsides.

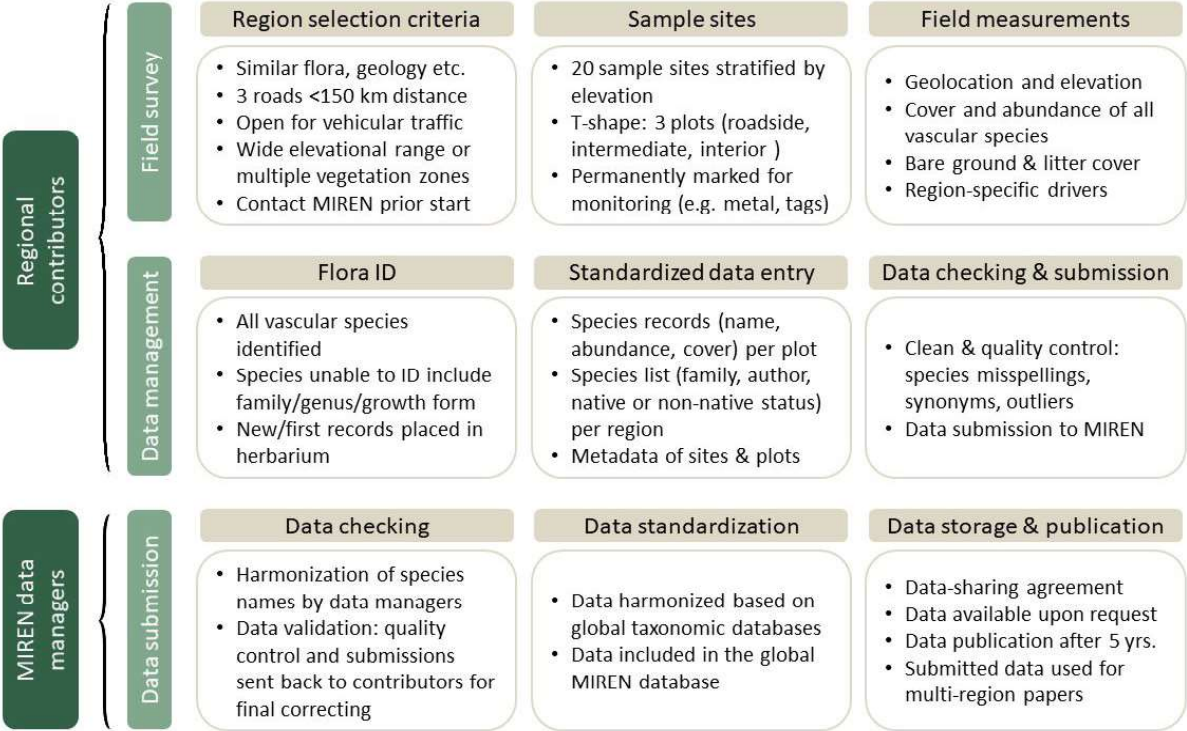


Figure 2. Overview of the workflow from region selection and data collection to inclusion of the data in the global MIREN database.

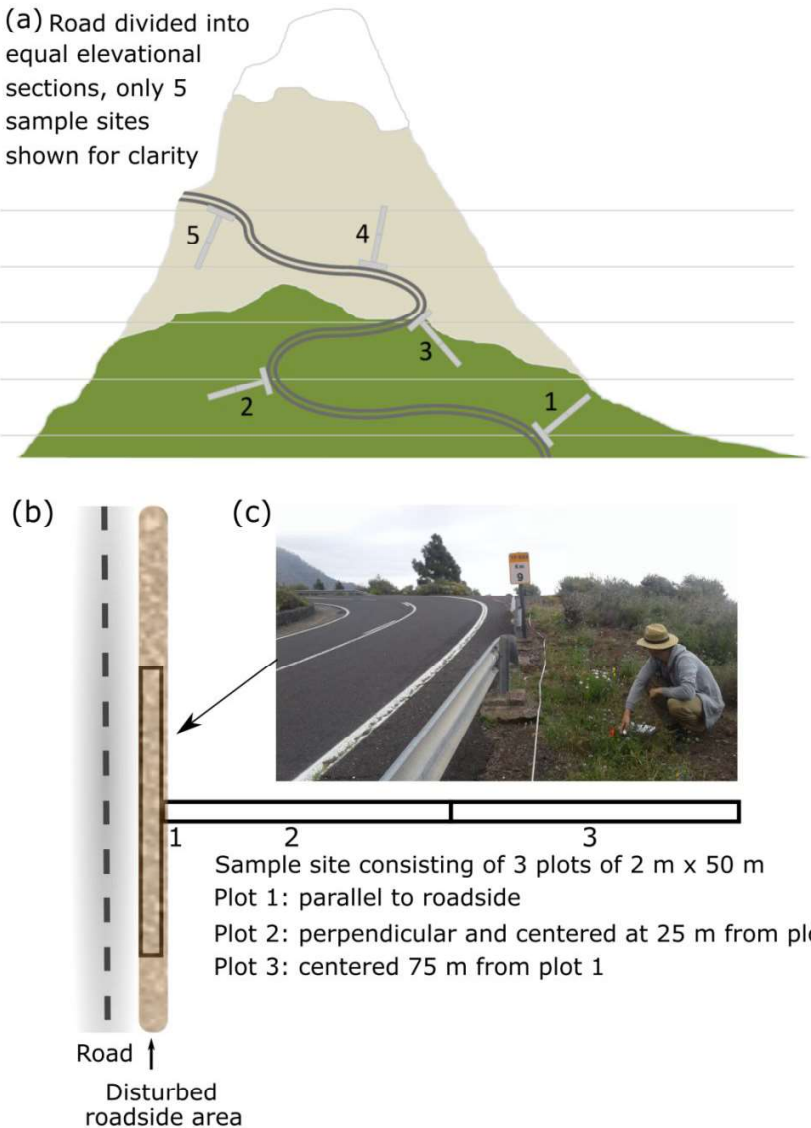


Figure 3: Layout of the MIREN sampling design. (a) Equal elevational distribution of 20 sample sites along a mountain road, of which three are selected in each region; (b) Each sample site consists of 3 plots of 2 m x 50 m, plot 1 – parallel to the roadside (starting at the first occurrence of roadside vegetation), plot 2 – centred 25 m from the roadside plot, plot 3 – centred 75 m from the roadside plot; (c) exemplary photograph of monitoring a mountain roadside in Tenerife, Canary Islands, Spain, depicting a survey of plot 1.

Repeated monitoring

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3 285 To understand long-term dynamics of redistributions of native and non-native plant species, all regions
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5 286 should strive for regular long-term monitoring, preferably with a periodicity of five years (and ideally with
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7 287 at least partial overlap in observers, to reduce observer bias). To facilitate monitoring, all plots should be
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9 288 permanently marked in the field, for instance with magnets or metal tags that can be relocated with a metal
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11 289 detector or coloured sticks or plastic seal security tags in remote areas where their removal is unlikely. In
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13 290 addition, precise sub-meter GPS coordinates should be taken at least once. Photographs should also be
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15 291 taken of each transect to visualize changes over time, document data collection and facilitate relocation of
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17 292 plots. Surveys and resurveys should always be done at peak biomass or flowering to minimize the risk of
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19 293 missing species with early or late phenology. For repeated surveys, this means that timing should be kept
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21 294 constant relative to the onset of spring, rather than to a fixed date, while sampling within season is
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23 295 recommended from valley bottom to top.
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28 297 *Add-ons to the standardized protocol*
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30 298 In addition to long-term monitoring of plant communities, the MIREN survey design is well suited for
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32 299 additional projects ('add-on' projects) that test more detailed or region-specific questions about the drivers
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34 300 of plant species redistributions. For example, soil temperatures have been recorded with a high temporal
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36 301 resolution for a year or longer in several MIREN regions to document how disturbance along roadsides
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38 302 affects microclimate, including consequences for species redistributions (for the first regional results see
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40 303 Lembrechts et al., 2019). Plant functional traits have additionally been collected for species in Tenerife,
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42 304 Canary Islands, to assess contrasting patterns of intraspecific trait variability of native and non-native
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44 305 species and the change of community mean traits and functional diversity with elevation (Kühn et al., 2020).
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46 306 Another add-on project has focused on soil chemical properties and mycorrhization of native and non-
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48 307 native species in the mountains of Norway (Clavel et al., 2021), and survey plots have also been used to
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50 308 assess the distribution of plant pathogens (*Phytophthora* species) in Australia (Khaliq, 2019). Once
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52 309 participants begin contributing data to the MIREN global road survey database, they can suggest add-on
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54 310 studies to apply across all regions that go beyond the existing scope of the survey protocol – as long as it is
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based on a standardized protocol that is fast, simple and low cost to implement by collaborators. To maximize participation and to discuss new proposals, data quality and complementarity, ideas for add-on projects should be developed together with the MIREN steering committee.

Data submission and accessibility

The MIREN survey design is a robust and standardized field survey protocol that provides data contributors amongst others an opportunity to include regional data in research that addresses globally scaled ecological questions. To be included in MIREN's global road survey database, regional data must be submitted to the MIREN data managers using a standard data format. An overview of how the database is structured and which metadata are stored is provided in Supporting Information S2). While in the first survey in 2007 only non-native species were monitored, we now only accept data submissions from new regions that surveyed all vascular plant species, both native and non-native, as such partial species pools drastically limit the amount of research questions that can be answered with the data.

All data will be made public in a data repository in the context of paper publications, or at the very latest five years after the survey is undertaken (Figure 2). To date, all survey data collected before 2016 are available through the Global Biodiversity Information Facility (GBIF; <https://www.gbif.org/publisher/76388ab6-61ca-439a-ab09-e1fe73eb224a>) and Zenodo (<https://doi.org/10.5281/zenodo.5529072>). Any researcher can also request the full MIREN database from MIREN data managers for global analyses. The structure of the database, with its plot-level table with accurate coordinates, and easy linkable species information, allows smooth integration into larger global integrative projects (e.g. data is currently integrated into the SoilTemp-database; Lembrechts et al., 2020). Details regarding data accessibility and publication, the submission of paper proposals and guidelines for co-authorship are given in MIREN's data-sharing agreement (see Supporting Information S3), which can also be downloaded from the MIREN website (www.mountaininvasions.org).

Results

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337 The standardized protocol for recording plant species communities along mountain roads has been
338 thoroughly tested in the field on all continents except Antarctica (Figure 4). The first survey was carried
339 out in eight regions in 2007 and has been repeated every five years since, resulting in one baseline historical
340 survey (2007) and up to two resurveys (2012 and 2017). The number of regions has increased since 2007,
341 with 18 regions performing the survey by 2018 (Figure 4). The global database currently includes circa
342 2,700 plots and >100,000 observations of >5,000 vascular plant species.

343 One of the most striking findings of the global MIREN surveys to date has been to document the importance
344 of roads in facilitating mountain invasions. Specifically, we found that non-native species richness in
345 roadsides decreases with increasing elevation, but generally peaks in the lower third of the elevation
346 gradient (Alexander et al., 2011). In a review including amongst others the MIREN road survey data, we
347 found that only 2.1% of the non-native species found in alpine areas can be considered as true alpine or
348 mountain plants based on their temperature affinity (Alexander et al., 2016). Moreover, the vast majority
349 of non-native species found at high elevation along the MIREN roads are also present at low elevation
350 (Alexander et al., 2011). These findings indicate that non-native species are first introduced and become
351 established at low elevation sites, following this they spread to higher elevations (Alexander et al., 2011).

352 At higher elevation sites, non-native species generally become increasingly filtered out by environmental
353 pressures, so few warm-adapted perennials and mainly generalist species reach higher elevations
354 (McDougall et al., 2018). Recently introduced species may also not have reached their elevational
355 maximum. However, a study from Switzerland demonstrated that non-native species did not rapidly expand
356 at their high elevation range limits over a period of six years (Seipel et al., 2016). We have also revealed
357 that the number of non-native species declines with increasing distance from the road (Seipel et al., 2012;
358 Haider et al., 2018), indicating that the native plant community serves as a second environmental filter that
359 selects for more shade- and moisture-tolerant perennials (McDougall et al., 2018). In addition to non-native
360 species, the MIREN surveys have shown that native species also use roads as corridors (Lembrechts et al.,
361 2017). Interestingly, Lembrechts et al. (2017) found that occurrence optima are higher in roadside habitats

362 than faraway habitats, and moreover that some alpine species have shifted their ranges downwards due to
 363 altered abiotic conditions and competitive release in roadside habitats (see also e.g. Lenoir et al., 2010).
 364 Globally, the MIREN surveys have demonstrated that native plant species richness does not follow a
 365 consistent pattern in non-roadside (semi-)natural habitat along elevation gradients, suggesting the existence
 366 of additional region-specific mechanisms, such as biome, vegetation type and human activity. These
 367 mechanisms are now the subject of further study. In contrast, a clearer elevation signal is present on roadside
 368 plots, with total species richness peaking at mid-elevations in most regions (Haider et al., 2018). Further,
 369 we have observed a reduction in community dissimilarity (beta-diversity) along roadsides relative to more
 370 distant plots, which is amplified by the arrival of non-native species along mountain roadsides
 371 homogenizing plant community composition (Haider et al., 2018). The MIREN surveys have also provided
 372 insight into the vulnerability of habitats regionally (Pollnac et al., 2012), the genetic background of
 373 successful invasions (Haider et al., 2012) and the impact and management of local invasions (McDougall
 374 et al., 2011a). For example, in the Greater Yellowstone Ecosystem in the United States, we found that non-
 375 native species emergence varies with elevation and habitat type, which provided land managers valuable
 376 information for mitigating biological invasions (Pollnac et al., 2012). Moreover, in the dry Mediterranean
 377 Andes in Argentina, which are characterized by treeless vegetation, the survey demonstrated how non-
 378 native plant species can successfully spread from the roadside into natural vegetation at low and
 379 intermediate elevations, thus highlighting the susceptibility of these types of ecosystems to invasion
 380 (Aschero et al., 2017). By contrast, the alpine vegetation of northern Norway has been shown to be more
 381 vulnerable to invasion than its low elevation counterpart, indicating that vegetation structure plays an
 382 important role in community invasibility (Lembrechts et al., 2014). Finally, the MIREN surveys have
 383 already generated information about regional floras. An excellent example is the discovery of a new species
 384 of Poaceae during MIREN monitoring in Kosciuszko National Park, Australia – this species was named
 385 after the network: *Poa mireniana* (Walsh & McDougall, 2018).

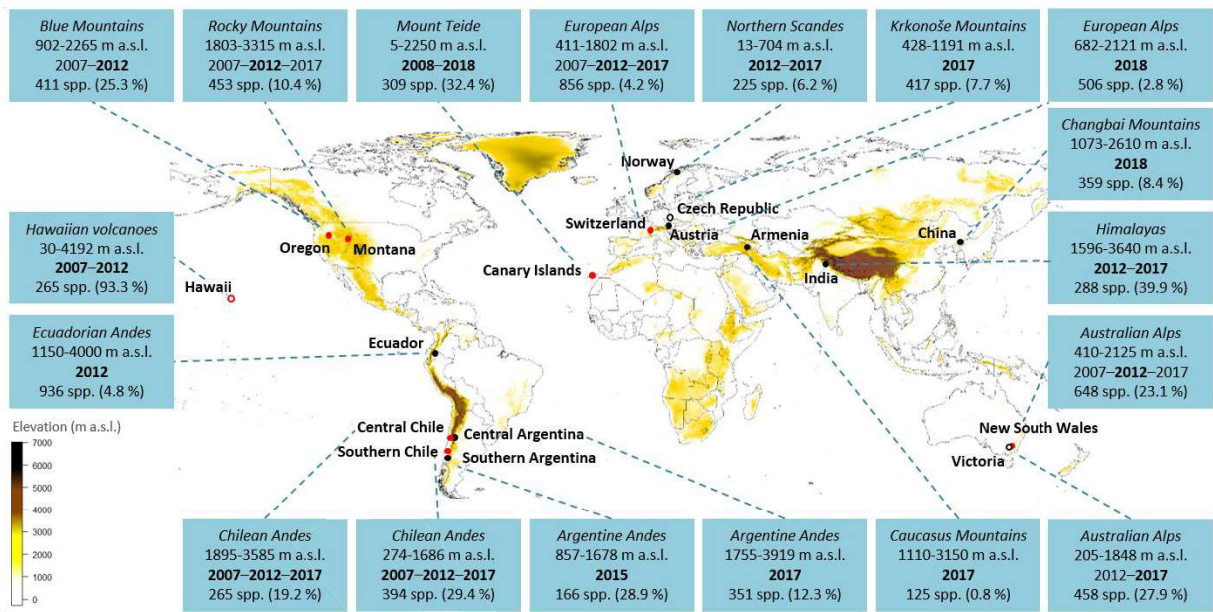


Figure 4: Regions worldwide participating in the vegetation survey along mountain roads according to the standardized protocol of the Mountain Invasion Research Network (MIREN). Red symbols indicate the founding regions from the first survey in 2007. In regions with unfilled symbols, only roadside plots, but not intermediate and interior plots in natural vegetation were sampled. For each region, the name of the mountain range, the sampled elevation gradient and the year(s) of sampling are given. Years in bold indicate that both native and non-native species were recorded, while in years with normal font only non-native species were recorded. Note that some regions did not follow the 5-year sampling frequency. In the last row, the total number of species and in parentheses the proportion of non-native species are summarized.

Discussion

Strengths of the protocol

The MIREN road survey protocol is unique for its focus on two critical co-occurring global change drivers on biodiversity and species redistributions in mountains: climate change and road construction (Figure 5). Road construction represents one of the most prominent and increasing land-use changes in many remote regions (Meijer et al., 2018), leading to physical disturbance, dispersal corridors and vectors for plant species (Gelbard & Belnap, 2003). Coupled with this, elevation gradients are good proxies for temperature

and can be used as space-for-time model systems for simulating climate change-induced temperature increase, where low elevation systems to a certain extent represent future scenarios for higher elevations in a warming climate (Blois et al., 2013; Lembrechts et al., 2017). Given this, combining elevation-based climate gradients with road effects allows researchers to disentangle the interactive effects of climate and road construction – as an example of human land-use change – on biodiversity, including their relative importance as drivers of species redistributions. Indeed, it is along clear linear dispersal pathways like roads that changes in species distributions – and especially those of non-native species – become apparent (Lembrechts et al., 2017). This is particularly relevant when considering the repeated survey approach of the MIREN design, which makes it possible to study the temporal dynamics of plant species distributions in response to natural (e.g. succession after natural disturbances, such as fire), as well as anthropogenic disturbances (e.g. land-use changes, such as increasing urbanization or domestic grazing, or the introduction of non-native species), allowing to assess how such disturbances affect the space-for-time proxy as would exist along gradients of climatic harshness only.

A final advantage is that along each road, sites are selected at predetermined elevations and capture all habitats found along an elevation gradient, equally covering all elevational belts. The protocol provides a methodological standardization that is straightforward to replicate globally and yet still yields sufficient explanatory power for regional case studies due to its relationship to the elevation gradient and its within-region replication (i.e. sampling along three mountain roads in each region; e.g. Arévalo et al., 2010; Pollnac et al., 2012). In doing so, the protocol remains simple, for example with plots close to roads remaining easy to reach, and thus applicable in many mountain regions even when fieldwork sites need to be easily accessible. This provides another strength of the protocol: it can be repeated in many places, so that general patterns at the global scale can be detected through multi-region replication (Alexander et al., 2011; Seipel et al., 2012; Lembrechts et al., 2017). In summary, data collected within the MIREN survey framework can be useful for regional and global studies in a large variety of fields, ranging from classical biogeography and community ecology to ecological modelling and global change research.

Strengths	Opportunities
• Strong environmental gradient • Natural vs. human-influenced habitats • Multi-scale approach • Long-term monitoring • Objectivity • Simplicity	• Global implementation • Links to other databases • Training tool (education) • Policy and management implications
Internal limitations	External limitations
• Most pristine communities excluded • Potential bias in vegetation types included • Detailed population information missed	• Short elevational gradients • Restricted permits and access • Funding insecurity

Figure 5: Summary of the strengths and opportunities of the MIREN road survey protocol as well as limitations of the protocol itself and those resulting from external circumstances.

Limitations of the protocol

The focus of the protocol on mountain roads provides excellent opportunities to disentangle the effects of climate and road construction on plant species and community redistributions. However, the protocol also has four important limitations, which we encourage users to keep in mind when applying the protocol to their study system (Figure 5).

First, the protocol excludes the most pristine environments that exist far from roads and at elevations above where roads reach, so does not monitor mountain biodiversity as a whole. As such, the protocol is a complement to the GLORIA protocol, which focuses on long-term climate change-related vegetation shifts on undisturbed mountain summits (Pauli et al., 2015). Nevertheless, one regional study has shown that, at least in northern Scandinavia, the effect of roads on mountain plant diversity could disappear beyond 25 m from the roadside (Lembrechts et al., 2014), and the direct impacts of road disturbance (e.g. construction, maintenance and vehicle use) are often firmly restricted to the road itself and its shoulders. This suggests

that the vegetation surveyed in the MIREN survey plot furthest from the roadside (Figure 3b; 50-100 m distance) may indeed at least be free of direct road effects. Yet, using these data beyond the 100 m reach of the sample site could bring issues for some applications, such as spatial modelling, where extrapolations for locations away from the road will suffer from increased uncertainty (Kadmon et al., 2004). Coupled to this, the restriction of the protocol to mountain roads means that, depending on the heterogeneity of the landscape, not all habitat types are necessarily covered relative to their distribution in the ecosystem. Plot locations may be biased towards valleys and less steep terrain if road construction favours such areas. Additionally, while roads represent the most prominent dispersal pathway present in mountains, they are not the only one (e.g. rivers, mountain trails, powerline cuttings, cable cars; Foxcroft et al., 2019). However, the protocol could be easily adapted for other pathways (as done for trails (Liedtke et al., 2020), railroads (Rashid et al., 2021) and rivers (Vorstenbosch et al., 2020)), and we suggest that this would be of particular interest in regions with sparse roads and/or where most of the common non-native species are wind or water dispersed.

Second, MIREN adopts a discrete temporal and spatial sampling approach. Specifically, since the protocol focuses on community dynamics and large-scale patterns it has a coarse spatio-temporal resolution, limited to monitor simple plant community composition estimates over time. The relatively low spatial sampling intensity (i.e. few plots for each elevational belt) and sometimes large distances between elevational increments (on average c. 75 m steps across current MIREN regions, but up to c. 160 m in the Indian Himalaya) can limit understanding of local processes, while also biasing sampling against rarer plant species or habitats. Furthermore, while repeated surveys facilitate investigation of species range dynamics under global change, the complete design does not explicitly consider dispersal dynamics (e.g. through seed rain or seed bank sampling, or seed tracking), instead assessing such dynamics indirectly through repeated snapshots of plant community composition. Additionally, with only one observational moment in a year, the phenological window of observation is small, with the risk of 1) excluding species flowering early or late in the season (Schultz et al., 2014), and 2) confounding phenological shifts over time with distributional

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3 470 shifts (CaraDonna et al., 2014). Whenever possible, we thus encourage regions to survey (a subset of) the
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5 471 plots at three time steps in the season. The latter would also allow for assessing detection probability.
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7 472 Third, the standard protocol emphasises simplicity to be as inclusive as possible and to keep resource use
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9 473 to a minimum. The approach thus focuses chiefly on plant community composition and coarse estimates of
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11 474 species abundance (see Supporting Information S1). Other important variables such as biomass, functional
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13 475 traits, community 3D-structure, species interactions and other abiotic and biotic variables thus require
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15 476 additional sampling effort. For the same reason, the protocol is limited to vascular plants, excluding
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17 477 bryophytes and other taxonomic groups of potential interest.
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19 478 Finally, the assumption that elevation can serve as a proxy for climate is of particular relevance here.
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21 479 Testing how the elevation gradient correlates with fine-grained climatic gradients requires validation using
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23 480 high-resolution climate data produced either using *in-situ* measurements or downscaling of climate models
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25 481 (Lembrechts et al., 2019). We therefore recommend participants to include at least one add-on study that
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27 482 deploys temperature data loggers to allow linking of vegetation patterns with microclimatic gradients
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29 483 (Lembrechts et al., 2019) – although this would already add cost.
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35 485 *External limitations*
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37 486 Application of the MIREN road survey protocol might be hindered in some regions, most obviously due to
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39 487 the lack of roads spanning sufficiently large elevation or climatic gradients. Additionally, local land
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41 488 ownership, safety issues or administrative complexities may complicate establishment and monitoring, for
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43 489 example on private land or in protected areas (Figure 5). Such issues might be of particular relevance in the
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45 490 MIREN survey design, as MIREN strives to cover a large elevation gradient spanning multiple vegetation
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47 491 zones. At the same time, the proximity of survey plots to roads increases the risk of damage over time (e.g.
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49 492 through road widening, mowing, pesticide use, expanding urbanization or occasional vandalism). The
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51 493 simplicity of the plot set-up nevertheless greatly reduces the impact of such damage or vandalism in the
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Long-term monitoring itself comes at a risk of funding insecurities, as the timeframe of 5-year intervals is beyond what is covered by most grants. Even though maintaining the observational sites themselves comes at virtually no financial cost, the monitoring involves considerable input of field labour, for which costs will vary between regions (Figure 5).

Opportunities

Many drivers of global change act rapidly and interactively, and intensify over time, so assessing their impact on global biodiversity urgently requires comparable data collected on a truly global scale. The MIREN road survey protocol has already demonstrated its potential to explain crucial patterns in native and non-native species redistributions along mountain roads, but there are a range of further applications that can be explored. For example, due to its simplicity the protocol can readily be implemented in many more mountain ranges and regions. Increasing the number of participating regions, all with their unique combination of climatic conditions and anthropogenic pressures, would further increase the potential to draw general conclusions about the interacting effects of climate change and roads as anthropogenic disturbance on mountain plant communities (Guo et al., 2018). This is particularly important for regions currently under-represented by the existing MIREN survey sites (Figure 4), such as Africa, Eastern Asia and central America, regions for which long-term biodiversity data are often lacking (Maestre & Eisenhauer, 2019). Despite these spatial gaps, MIREN has already more than doubled in size on its road to becoming a global-scale network since it was first established in eight regions. New participants would thus be able to place their region into a much larger spatio-temporal picture and, as time passes, get an increasingly strong grasp of how species distributions are changing dynamically, regionally and across the world.

With its potential to answer important local questions, and feed into the growing multi-region database, we hope that the MIREN road survey protocol will become the protocol of choice for those interested in native and non-native plant biodiversity dynamics in mountain regions. At the local scale, it can provide good baseline data on biodiversity changes along elevation gradients in disturbed regions, with opportunities to

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3 521 inform management decisions (McDougall et al., 2011a). For example, it can inform policy makers on some
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5 522 of the impacts of urban expansion and new infrastructure projects in mountains, as well as identify new
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7 523 non-native species before they become problematic. The protocol can also provide essential biodiversity
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9 524 variables for global monitoring efforts (Jetz et al., 2019), since it provides insight into species abundance
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11 525 change over space and time and can further enrich the mountain biodiversity data provided on the online
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13 526 data portal of the Global Mountain Biodiversity Assessment (GMBA). In doing so, it has the capacity to
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15 527 inform global biodiversity policy initiatives, such as the Intergovernmental Science-Policy Platform on
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17 528 Biodiversity and Ecosystem Services (IPBES).
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19 529 Further opportunities include add-ons and expansions to the protocol design, for example to measure
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21 530 microclimate (Lembrechts et al., 2019), dispersal dynamics (e.g. with seed traps), soil biodiversity (e.g.
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23 531 analyses of the soil microbiome or mycorrhizal colonization of roots) or plant-animal interactions (e.g.
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25 532 pollinator records, herbivore abundance). Collecting such data would be important not only in isolation, but
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27 533 also for helping to create explicit links between descriptive and predictive species distribution models, both
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29 534 at local and global scales. Such efforts could even facilitate modelling of (changes in) the distributions and
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31 535 habitat occupation of mountain plant species, for instance by coupling georeferenced long-term survey plots
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33 536 with high-resolution remotely sensed and modelled environmental data (Randin et al., 2020). The survey
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35 537 approach can similarly be expanded by adapting it for use along other linear introduction pathways for non-
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37 538 native species, such as rivers or hiking trails, or by connecting it with other standardized global biodiversity
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39 539 surveys and assessments, such as GLORIA (Pauli et al., 2015), sPlot (Bruehlheide et al., 2019), the Global
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41 540 Inventory of Floras and Traits (GIFT; Weigelt et al., 2020), the Global Naturalized Alien Flora (GloNAF)
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43 541 database (van Kleunen et al., 2015) and the BioTIME database (Dornelas et al., 2018). The connection to
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45 542 other datasets can be done either via the exact geographic location submitted with the MIREN plot-level
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47 543 data, or via the species names which have been standardized with major taxonomic backbones, such as
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49 544 World Flora Online (<http://www.worldfloraonline.org>). Finally, the protocol has already shown to have
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51 545 great potential for teaching, for instance by training undergraduate and graduate students in vegetation
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sampling, while also having relevance for local policy and management, for example as demonstration sites (Figure 5).

Conclusions

The MIREN road survey protocol started in 2007 with a specific purpose – to monitor non-native plant species invasions along mountain roads – but has since then proven to be well-suited for an increasing number of questions related to species redistributions in the fields of biogeography, ecology and conservation biology. The protocol is low-tech, straightforward and standardized, and can therefore be implemented immediately to fill global gaps in biodiversity data, especially in areas that are traditionally underrepresented in global biodiversity studies (Nuñez et al., 2019) or in regions with scarce or fluctuating government support for scientific research. In short, this on-the-ground, multi-regional, simple yet effective monitoring scheme is a perfect example of ‘Think globally, measure locally’, and has clear capacity to bring together ecologists from around the world to generate an even more complete picture of ongoing species redistributions in mountains. We invite you all to join us!

Acknowledgements

We gratefully acknowledge the immense effort and time that numerous professionals, field assistants, and students have contributed to the collection of these data. SH and ARB gratefully acknowledge the support of iDiv funded by the German Research Foundation (DFG–FZT 118, 202548816). JJL received a postdoctoral fellowship from the Research Foundation Flanders (FWO, grant nr. 12P1819N). The work from JJL, JC and IN is supported by a research grant from the FWO (grant nr. G018919N). Fieldwork in the Northern Scandes is achieved using INTERACT Transnational Access funding. LAC and AP acknowledge support from FONDECYT 1171005, 1180205, CONICYT PIA CCTE AFB170008 and Grant ANID PIA/BASAL FB210006. JMA received funding from the European Commission Horizon 2020 research and innovation programme under grant agreement 678841. VA was supported by the Universidad Nacional de Cuyo (SIIP 2019 UNCUIYO). FE, BL and TV acknowledge funding by the Austrian Science

1
2
3 572 Foundation FWF (grant I 3757-B29). OG received a postdoctoral research fellowship through the
4
5 573 Afromontane Research Unit, University of the Free State. PK acknowledges support from the Swedish
6
7 574 Research Council (Vetenskåprådet) (project grant 2015-04214). BN was supported by the US Forest
8
9 575 Service and direct mentorship from C. Parks. VS was supported by SENESCYT-Ecuador, Instituto de
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11 576 Ecología y Biodiversidad (IEB) and Laboratorio de Ecología, Biogeografía y Sistemática (ECOBIOSIS) de
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13 577 la Universidad de Concepción, Laboratorio de Invasiones Biológicas (LIB) de la Universidad de
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15 578 Concepción, Herbario QCA and QCNE-Ecuador, Missouri Botanical Garden, and CONICYT (PFB-023).
16
17 579 KLS thanks funding received from PICT 2015-2072 and PIP 2014-0758. MV was supported by a research
18
19 580 grant from the BiodivClim Call 2019 (grant nr. TACR SS70010001) and together with JM by long-term
20
21 581 research development project RVO 67985939 from the Czech Academy of Sciences. We also thank the
22
23 582 Fondation Herbette, University of Lausanne, for financial support to JMA for the workshop “Distributed
24
25 583 ecological observatories and long-term monitoring of mountain biodiversity” held in September 2019 that
26
27 584 lead up to this paper. Finally, we are grateful to the editor and two anonymous reviewers for providing
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29 585 helpful comments to improve the manuscript.
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35 587 **Authors’ contribution**

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37 588 This paper builds on the protocol as designed originally by the Mountain Invasion Research Network
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39 589 (MIREN) by SH, KM, AP, JMA, LJR, JRA, CD, HD, PE, GJ, CK and BN. The idea for the paper and
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41 590 further fine-tuning of the protocol stems from a MIREN workshop in 2019, attended by SH, KM, AP, JMA,
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43 591 AB, LAC, CC, VRC, AG, AH, PK, CK, IR, ARB, SR, DU, VV, TWNW, SZ, and TS. SH, JJJ and TS led
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45 592 the writing of the manuscript, with contributions by the MIREN steering committee (KM, AP, JMA, AB,
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47 593 LAC, IR, LJR). All authors contributed critically to the drafts – based on their experience in applying the
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49 594 protocol in their regions – and gave final approval for publication.
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54 596 **Data availability**
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Data collected according to the MIREN road survey protocol will be published in the context of paper publications or five years after data collection. Details are governed in the current version of the MIREN data-sharing agreement which is available on the network's website (www.mountaininvasions.org) and provided as supplementary material to this manuscript. MIREN road survey data from 2007 to 2015 are available at Zenodo (<https://doi.org/10.5281/zenodo.5529072>) and GBIF (<https://www.gbif.org/publisher/76388ab6-61ca-439a-ab09-e1fe73eb224a>)

Supporting Information

Supporting Information S1: MIREN road survey protocol

Supporting Information S2: Structure of the global MIREN road survey database

Supporting Information S3: MIREN Data-sharing agreement

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Figure 1: Examples of roads in the landscape (a-c) and key non-native species (d-f) across a range of MIREN regions. (a) Harsh mountain climates (here the Cañadas del Teide on Tenerife (Canary Islands, Spain) have traditionally been seen as an adequate barrier against non-native plant invasion; (b) the direct local impact of roadside disturbance on mountain plants is visible on native *Azorella* cushion plants along a road in the dry Andes near Mendoza, Argentina; (c) interactive effects of climate and land use, exemplified by dramatic differences in snow cover on versus beside a mountain road in northern Norway; (d) *Taraxacum officinale*, one of the most widespread non-native plant species along MIREN mountain roads (Seipel et al., 2012), in a sample plot on a volcanic gravel slope in the Argentine Andes; (e) non-native *Verbascum thapsus* on a roadside in the highly invaded lowlands of the Andes in central Chile; (f) *Trifolium pratense* in northern Norway, where the species is rapidly moving uphill along mountain roadsides.

590x564mm (59 x 59 DPI)

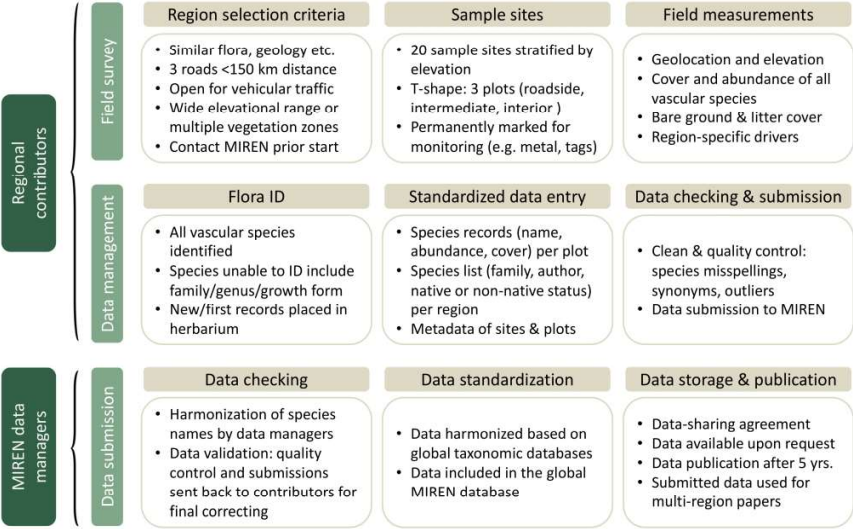


Figure 2. Overview of the workflow from region selection and data collection to inclusion of the data in the global MIREN database.

861x484mm (118 x 118 DPI)

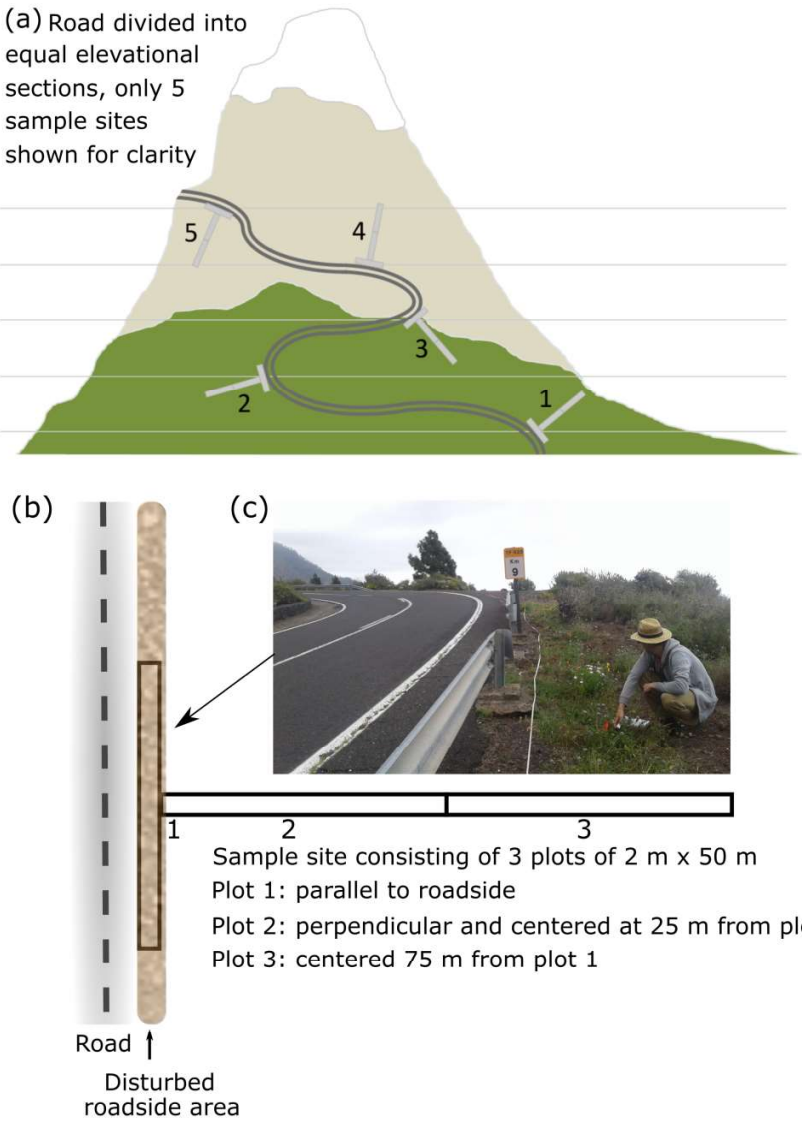


Figure 3: Layout of the MIREN sampling design. (a) Equal elevational distribution of 20 sample sites along a mountain road, of which three are selected in each region; (b) Each sample site consists of 3 plots of 2 m x 50 m, plot 1 – parallel to the roadside (starting at the first occurrence of roadside vegetation), plot 2 – centred 25 m from the roadside plot, plot 3 – centred 75 m from the roadside plot; (c) exemplary photograph of monitoring a mountain roadside in Tenerife, Canary Islands, Spain, depicting a survey of plot 1.

546x733mm (118 x 118 DPI)

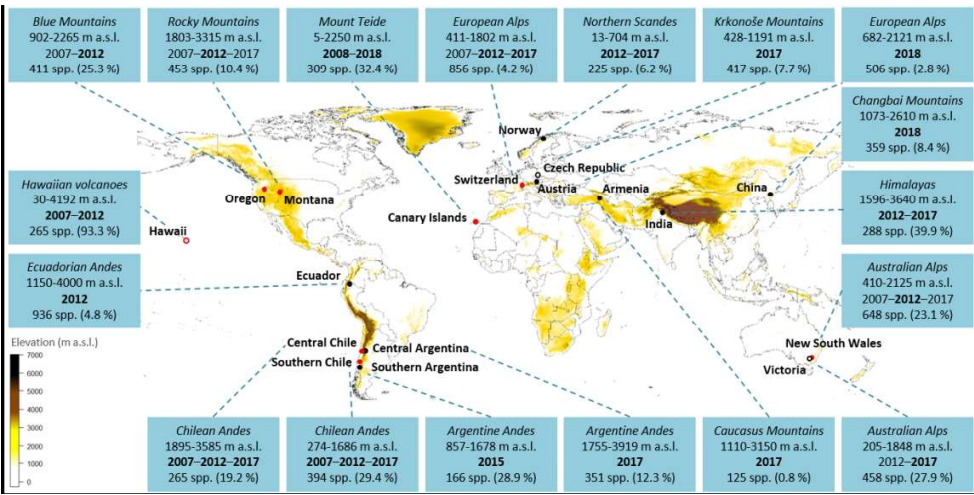


Figure 4: Regions worldwide participating in the vegetation survey along mountain roads according to the standardized protocol of the Mountain Invasion Research Network (MIREN). Red symbols indicate the founding regions from the first survey in 2007. In regions with unfilled symbols, only roadside plots, but not intermediate and interior plots in natural vegetation were sampled. For each region, the name of the mountain range, the sampled elevation gradient and the year(s) of sampling are given. Years in bold indicate that both native and non-native species were recorded, while in years with normal font only non-native species were recorded. Note that some regions did not follow the 5-year sampling frequency. In the last row, the total number of species and in parentheses the proportion of non-native species are summarized.

236x118mm (150 x 150 DPI)

Strengths	Opportunities
• Strong environmental gradient • Natural vs. human-influenced habitats • Multi-scale approach • Long-term monitoring • Objectivity • Simplicity	• Global implementation • Links to other databases • Training tool (education) • Policy and management implications
Internal limitations	External limitations
• Most pristine communities excluded • Potential bias in vegetation types included • Detailed population information missed	• Short elevational gradients • Restricted permits and access • Funding insecurity

Figure 5: Summary of the strengths and opportunities of the MIREN road survey protocol as well as limitations of the protocol itself and those resulting from external circumstances.

861x484mm (118 x 118 DPI)

Road Survey Protocol of the Mountain Invasion Research Network

Date of this version: 2021-11-21

For more information see www.mountaininvasions.org



1 Overview of the MIREN road survey protocol and contributions

The aim of the Mountain Invasion Research Network (MIREN) road survey protocol is to investigate the changing distribution of native and non-native vascular plant species along elevation gradients in mountain regions around the world. MIREN focuses on the detection of species redistributions due to drivers of global change, such as climate and land-use. Data generated using the standardized protocol described in this document can be used to evaluate and quantify the processes and mechanisms shaping mountain plant communities at regional to global scales. We encourage implementation of the protocol in mountain regions across the globe. For more information about this protocol please also see Haider et al.¹

2 Protocol methodology

The MIREN road survey uses a stratified approach for recording plant species along mountain roads that traverse the major elevation gradient in a mountainous region (Fig. 1). Stratified sampling occurs within a **Region** along three different **Roads**. Along each Road there are 20 **Sites** evenly stratified by elevation, and at each Site there are three **Plots** at different distances from the road.

2.1 Regions

The delineation of a region will depend on many local factors and we do not seek to constrain these. However, a region must be mountainous as defined by Körner et al. (2017)² or the Global Mountain Biodiversity Assessment (GMBA; www.mountainbiodiversity.org). Ideally, a new region should not be in, or part of, an existing region (see www.mountaininvasions.org for an up-to-date map). If you are in doubt about whether your region is suitable for inclusion in the MIREN database (see section 2.6.1), please contact MIREN (miren.contact@gmail.com).

¹ Haider S., Lembrechts J.J. et al. (submitted). Think globally, measure locally: The MIREN standardized protocol for monitoring species distributions along elevation gradients.

² Körner C. et al. (2017). A global inventory of mountains for bio-geographical applications. *Alpine Botany* 127, 1–15. <https://doi.org/10.1007/s00035-016-0182-6>.

2.2 Roads

A road is defined as a corridor which is open to motorized traffic at some point during the year, and which is *not* only used for livestock or as a hiking path. It may be a single corridor or part of a network of corridors. However, each road must continuously connect low and high elevations. Three roads are sampled in each region. Where a choice of roads exists, choose roads which 1) have the largest elevation range, 2) have the most traffic, and 3) capture the geographic and/or environmental variation within the region. Roads should cover a wide elevational range or traverse multiple vegetation zones, ideally including the alpine zone. Defining the bottom of a road is left to the discretion of each group but could be sea level, a place where there is no longer a significant change in elevation, a management boundary (e.g. conservation reserve), or beyond which it is impractical to sample (e.g. urban center). The highest point of the survey should be the highest elevation reached by the road (or accessible by motorized traffic).

2.3 Sites

Sample sites along each road should be evenly stratified by elevation (Fig. 1a). Starting at the bottom, the elevation range of each road is divided into 19 equally spaced bands using digital elevation models or topographic maps, giving 20 sample sites per road. Sample sites are determined *prior* to going into the field to avoid bias, and subsequently located using a global positioning system (GPS). Sites are numbered by elevation along each road from lowest (01) to highest (20). Choose the side of the road to place sample plots at random unless only one side is possible.

When in the field, confirm that the sample site meets the following criteria:

- a. The furthest measurements will be required at least 100 m from all roads, so avoid switchbacks, proximity to secondary roads and impassable barriers, e.g. cliff, large river, etc.;
- b. It is safe to make measurements at the location (e.g. not on the bend of a busy, narrow road).

2.4 Plots

Each sample site consists of three plots (Fig. 1b), one plot parallel to the roadside (plot 1) and two plots perpendicular to the roadside plot. The intermediate plot (plot 2) adjoins the middle of the roadside plot forming a “T”. The interior plot (plot 3) abuts plot 2 and ends 100 m from the roadside plot (Fig. 1b). Each plot is 2 m x 50 m. The edge of the roadside plot closest to the road should be placed where roadside vegetation begins and sampling can be safely conducted. If time or resources do not permit three plots to be sampled at each site, preference should be given to the roadside plot (plot 1) and the interior plot at 50-100 m from the roadside (plot 3).

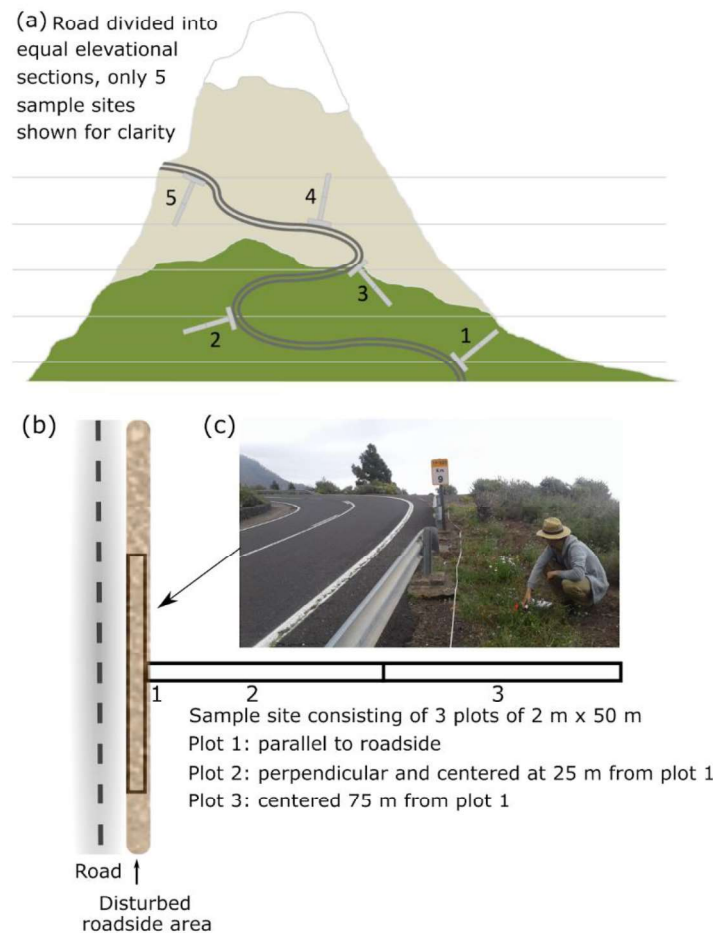


Figure 1. Layout of the MIREN sampling design. (a) Equal elevational distribution of 20 sample sites along a mountain road, of which three are selected in each region; (b) a site consisting of 3 plots of 2 m x 50 m, starting in the disturbed roadside area and extending perpendicularly into the natural vegetation; (c) monitoring of plot 1 in a mountain roadside in Tenerife.

All plots should be permanently marked in the field to facilitate monitoring. Example marking techniques include magnets that can be relocated with a metal detector, colored sticks in remote areas where their removal is unlikely, metal stakes, plastic seal security tags or if physical markers are not possible a combination of hand drawn maps and photos. Very accurate GPSs can also be used to relocate markers. To visualize changes over time and facilitate relocation, photographs should be taken of each plot, ideally with the plot markers visible. This will be especially important for plot 1 (roadside) because the proximity of vegetation to the road may change over time with disturbance. The location of each photo point should be recorded so repeat photos can be taken.

2.5 Data collection

The following descriptions are based on the criteria to include a dataset into the global MIREN database (see section 2.6.1).

All data sheets must include date of survey and names of recorders to facilitate data checking. If you have any questions regarding data collection, contact miren.data@gmail.com.

2.5.1 Site measurements

- *Geolocation* should be recorded at a minimum for each site at the center of the roadside plot (plot 1). It is helpful to record location ends of plots 2 and 3 at 50 and 100 m but this may not be possible if plots 2 and 3 are heavily forested. Geolocation should be reported as latitude and longitude in decimal degrees using the WGS 84 datum; please check that the mapped locations are correct using GIS or Google Earth before submitting data.
- *Elevation*: in meters above sea level; obtained using a digital elevation model or Google Earth; at the same locations as *geolocation* is recorded.

2.5.2 Plot measurements

The following is to be recorded in each 2 m × 50 m plot:

- *Plot code*: is the unique identifier for each plot and is recorded in the form **Region.Road.Site.Plot** (e.g. MTN.BP.06.2). Region should be three letters (e.g. MTN = Montana), and Road a two letter code (e.g. BP = Beartooth Pass Road). Region and Road codes may be altered by the database managers if they have already been used elsewhere. Sites are numbered from 01-20 with 01 being the lowest and 20 the highest. Please include the 0 as a place holder for site codes to ensure character strings of the same length. The roadside plot is numbered 1, the intermediate plot is numbered 2 and the interior plot is 3. If the intermediate plot is not sampled the interior plot should still be recorded as 3.
- *Name and cover of each species*: see section 2.5.2.1 for species nomenclature information and see section 2.5.2.2 for the ordinal projective foliage cover estimates to be used.
- *Abundance of each species* on the following scale: 1 = 1 to 10 individuals (or ramets); 2 = 11 to 100 individuals; 3 = >100 individuals. This coarse scale was used in initial samplings of the global database and its continued use will ensure comparability with previous sampling times.
- *Tree cover*: percent of plot covered by trees taller than 3 m.
- *Bare ground*: percent of plot without vegetation foliage (but excluding rock and litter).
- *Litter*: percent of plot without vegetation foliage but covered with dead and decaying plant material (such as leaves, bark, needles, and twigs)

- *Additional data (optional)*: these can be specific disturbance types, land-use, habitat type, cover of rock, or any other descriptive factors. The data should be provided as text (up to 50 characters) with words separated by an underscore.

2.5.2.1 Species information

All angiosperms, gymnosperms and ferns, both native and non-native, that create foliar cover in a plot are to be recorded. Mosses and lichens can be recorded for regional purposes but are *not* to be submitted to the global database.

If a species can only be identified to family or genus level, a region-specific name should be given. This name is a combination of the taxonomic level, a three-letter region code, and a number (e.g. Acacia MTN1 or Asteraceae MTN2). If species cannot be identified to family level, the name should consist of life form (either graminoid, herb, fern, vine, shrub or tree), the region code and a number (e.g. shrub ORE3).

2.5.2.2 Projective foliage cover estimates

Estimate projective foliage cover of each plant species using the following scale. Projective foliage cover is defined as the proportion of the ground that is shaded by vegetation foliage when lit from directly above. Note that the sum of all species-specific cover estimates can exceed 100 % due to overlap in coverage.

Cover class	1	2	3	4	5	6	7	8
Percentage area	< 0.1	0.1 - 1	2 - 5	6 - 10	11 - 25	26 - 50	51 - 75	76 - 100
Indicative max. area (m x m)	0.1 x 1	1 x 1	1 x 5	1 x 10	1 x 25	1 x 50	1.5 x 50	2 x 50

2.5.3 For repeated observations

For regions repeating the MIREN survey (see also section 2.6.2), relocate the plots used in the previous survey, resample at the same time of year and take new photos. Where it is not possible to resample the same site (e.g. where a major disturbance destroyed the vegetation), establish a new site nearby, and identify the change in the plot code by adding an 'a' to the end of the plot code (e.g. MTN.BP.06.2a). When submitting the data inform the data managers that this is a new site. To avoid bias, we suggest sampling without the records from previous years. However, after finishing a plot in the field, the new species records should be compared with the old survey(s) to avoid missing rare species that were recorded previously, and to be consistent in species identification (especially if different people did the previous survey).

2.5.3.1 *Survey timing*

Sampling should be done at a time that maximizes the chance of recording all species present. This will often be during the peak of flowering or biomass production; i.e. when most species are identifiable. Since flowering at high elevation is often later than at low elevation, it may be expedient to conduct sampling when the highest plots are at their peak of flowering; at that time, low elevation plots will typically be commencing seed production but still identifiable.

2.5.3.2 *For repeated observations*

For regions repeating the MIREN survey (see also section 2.6.2), relocate the plots used in the previous survey, resample at the optimal time of year for species identification and take new photos. This means that timing should be kept constant relative to the onset of spring, rather than to a fixed date.

Ideally, there is at least partial overlap in observers across survey, to reduce observer bias. Additionally, it is recommended that observers take species lists of previous survey(s) to the field, to assess immediately if species were missed or misidentified. To prevent additional bias emerging from such an approach, we suggest to do a 'blind' sampling, and to compare the new species list only afterwards (but immediately afterwards, to allow repeat recording and corrections).

Whenever possible, regions are encouraged to survey (a subset of) the plots at three time steps in the season to allow for assessing detection probability.

2.6 Data management

Data submitted to the global MIREN database include three spreadsheets:

- (1) Species records with the following column headings: a) unique identifier for each plot, b) species name, c) abundance score and d) projective foliage cover class.
- (2) A list of all species recorded in each region including family, author and native / non-native status in that region, with data sources for native / non-native status. If the native / non-native status is not known or ambiguous, it should be recorded as 'unknown'.
- (3) A metadata-file including all site- and plot-level data listed above.

Templates for these three files can be found on www.mountaininvasions.org or requested from the data managers (miren.data@gmail.com) and *must* be used for submitting data.

A stringent quality control of spelling and synonymy must be undertaken. All species names should be standardized according to the Taxonomic Name Resolution Service (TNRS; <http://tnrs.iplantcollaborative.org/>) or, if not recorded there, according to a modern, cited flora. Datasets that do not comply with formatting rules will not be incorporated and authors will be notified about which changes are required.

Data should be sent to miren.data@gmail.com.

2.6.1 Inclusion in the MIREN database

Although there is no requirement that data collected using the MIREN road survey protocol is contributed to the MIREN database, we strongly encourage submission of regional datasets. The requirements for data submission are outlined in the MIREN data-sharing agreement document available through www.mountaininvasions.org. Acceptance of data into the database will be at the discretion of the data managers, according to how closely the region has followed the protocol. We strongly advise regions aiming to have their dataset included in the global database, but unable to conduct the complete survey, to contact the data managers *before* starting with the survey. As of January 2021, the MIREN database comprises >100,000 records of >5,000 species in 18 mountain regions around the world.

2.6.2 Observations over time

Some regions have monitored the same plots since 2007 by repeating the survey every five years, enabling evaluation of temporal dynamics. Regardless of when a region does the first survey, to aid data management and preparation of papers using the global dataset we ask that all subsequent surveys be done concurrently: the next repeat survey in the southern hemisphere will be in 2021/22, and in the northern hemisphere in 2022, and then every 5 years.

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Supporting information S2: Structure of the global MIREN road survey database.

The core database consists of three tables which can be linked to each other. Table A gives information about the cover and abundance of each species in each plot. Through the combination of species name and region, this table can be matched to table B, which provides the status (alien, native, unknown) for each species in the respective region. Table C contains information at the plot or site level, such as geolocation and basic environmental variables like tree cover (indicted here as Envir1 etc.). While some of the information are constant over years (e.g. geolocation), other variables might change with time (e.g. cover of bare soil). Therefore, all three variables of region, plot ID and year have to be used to match tables A and C.

Further species information from add-on studies (e.g. functional traits or mycorrhizal association) can linked easily through table B. Additional plot information (e.g. soil properties or in-situ temperature measurements) can be linked via table C.

This structure has been proven very practical because it does not require from data contributors to be familiar with specific database programs, and for sub-setting and data analyses the tables can simply be handled in the open source statistical environment of R.

(A) Species cover and abundance

Spec.	Region	Plot ID	Year	Cover	Abund.

(B) Species status

Spec.	Family	Region	Status

(C) Environmental data (plot information)

Region	Plot ID	Year	Lat	Long	Elev.	Envir1	Envir2	...

(D) Additionally, we collect metadata per region, which has to be submitted with every (repeated) survey. Metadata is collected primarily as background information and not for analyses. Therefore, they are stored in a separate table D and not included in table C. However, via the variables of region, plot ID and year, metadata can easily be matched to tables A and C. Besides information about data sampling (observer and date), we store information about the regional literature used to determine the species status (see table B) and if plots are located in protected areas (PA).

(D) Metadata (plot scale)

Region	Plot ID	Year	Observ.	Date	Literat.	PA

Data-sharing agreement relating to the use of data collected by the Mountain Invasion Research Network

Target: Data collected according to the MIREN road survey protocol

Version 1.0

Approved by the MIREN Steering Committee 2020-04-19

For more information see www.mountaininvasions.org



1. Data submission

- (a) Data submitted to the global MIREN road survey database must be provided in the standardized format created by MIREN. A template is available on www.mountaininvasions.org/?page_id=688 or through an e-mail to miren.data@gmail.com.
- (b) Data must be submitted to the MIREN data managers (miren.data@gmail.com).
- (c) Data must be cleaned and checked, and will not be accepted if these requirements are not fulfilled.
- (d) With submission of their data, the contributing region
 - nominates 1 or 2 contact persons recognized as owners of the data, that therefore have the right to submit the data to a global database. These persons will be the “data owners”.
 - agrees that the regional data will be harmonized (especially species taxonomy) according to the existing procedure developed by the MIREN data managers based on global taxonomic names.
 - agrees that the MIREN Steering Committee has authority to decide whether data from multiple MIREN regions can be used for analyses, based on 1-page proposal to the MIREN Steering Committee.
- (e) Submitted data will be used in multi-region analyses.
 - The regions give their consent that their data can be used for papers lead by other MIREN data contributors. If a region’s data is used, the regional data owners will be informed by the MIREN Steering Committee. Data contribution will be recognized according to “5. Co-authorship”.
 - For accepted paper proposals of non-data contributors (third parties), the data managers will ask the region for data use permission and provide the opportunity to opt out.
 - The data managers will never hand over regional data for a single-region paper (data owners can of course use their own data).
- (f) Corrections of original datasets must be sent to the MIREN data managers.
- (g) Regional datasets may be withdrawn from the global dataset at any time before the data has been published. Already approved paper proposals may still include these data in their analyses. The MIREN Steering Committee decides if a once withdrawn dataset might be re-integrated.

2. Data publication

- (a) The submitting region agrees that (part of) their data are published as required by journals for paper publications. This can be only species occurrence data with point location (to be published in GBIF; www.gbif.org), or the full dataset used in the analysis (to be published in Dryad; www.datadryad.org).
- (b) Time of data publication:
 - Species occurrences with point locations (GBIF): earliest publication is 2 years after data were collected. If the first paper is published earlier, the lead author should ask the journal for a moratorium.
 - Full dataset used in the analysis (Dryad): In any case, the lead author should ask the journal for a moratorium of 2 years after paper publication to allow time for other papers to be published.
- (c) The submitting region agrees that independent of paper publications all species occurrence data with point location will be submitted to GBIF 5 years after data were collected.

3. Requests for MIREN road survey data - Paper proposals

- (a) The global MIREN road survey dataset might be used by data contributors as well as non-data contributors (third parties). In both cases, a paper proposal needs to be sent to the MIREN co-chairs (miren.chairs@gmail.com; see also www.mountaininvasions.org/?page_id=6).
- (b) The paper proposal needs to contain the following (the standardized template created by MIREN must be used):
 - Preliminary title
 - Brief outline with aims and methods of the study (approx. 0.5 pages)
 - A list of authors that will be working with the data, especially those people coming from outside MIREN
 - Define a rough timeline
 - Specification of required data (which regions, plots, species, years)
 - Explicit statement that the applicant agrees with this data-sharing agreement
- (c) The MIREN Steering Committee will decide within a month, if the paper proposal is approved, based on the following criteria:
 - There is a reasonable link between the aims, expected outputs and data requested.
 - There is no conflict with ongoing publication projects using the MIREN database.
 - This data-sharing agreement is respected.
- (d) The titles of approved paper proposals, together with the names of their applicants, might be published on the MIREN website and in the MIREN newsletter.
- (e) If the paper applicant is a data contributor, the data managers will prepare the dataset needed for the planned analyses and in parallel the regions whose data will be used will be informed about the planned paper by the MIREN Steering Committee.
- (f) If the paper applicant is not a data contributor, the MIREN Steering Committee will ask all regions whose data were requested, if they are willing to share their data for the specific proposal (opt-out option). Given their agreement (or denial), the data managers will finalize the data set needed for the planned analyses.

- (g) After the approval of a paper proposal, the lead author needs to give a short progress report after 6 months. If after 1 year no evidence of progress is given, the applicant needs to ask for proposal renewal, otherwise the proposal is no longer approved, the right to use the data has expired and the topic is open for others.

4. Regional analyses and papers

- (a) Regional data may of course be used for regional analyses and papers. However, for approved paper proposals, the regions are strongly encouraged to submit regional papers based on the same main idea only after the global paper is accepted by a journal.

5. Co-authorship

- (a) Opt-out: Each region whose data is used in a paper can nominate one person per survey year as a co-author to acknowledge data contribution (i.e. 1 co-author for the first survey, 2 co-authors for the second survey etc.). Additionally, co-authorship has to be offered to the data managers.
- (b) Opt-in: Co-authorship can be earned through substantial intellectual contribution. After approval of a paper proposal and, if needed, approval of data sharing, the MIREN Steering Committee informs the wider MIREN group about the planned analyses. At this point of time, people can state within 4 weeks their interest in becoming a co-author by sending a short statement to the Steering Committee about how they will contribute.
- (c) All co-authors should receive at least three opportunities to contribute and review the manuscript. Typically, this should be at the beginning of the project, at the time of the first full manuscript draft, and before paper submission. Opt-in co-authors are expected to contribute at all three stages. Opt-out co-authors are expected to, at least, review and explicitly approve the publication. If a data contributor does not respond to emails approving the final publication, he/she will be removed from the author list (the dataset remains included).
- (d) The rules described above are mandatory for all papers based on unpublished data at the time of paper proposal approval. For papers using published data (published full data sets), it is recommended to follow the same criteria.
- (e) In any publication making use of MIREN data, the applicants are obliged to give appropriate credit to MIREN. Upon publication, the applicants are requested to send the paper(s) based on MIREN data to the MIREN co-chairs.

6. Personal data

- (a) According to the General Data Protection Regulation (GDPR; <https://gdpr-info.eu>) that came into force on 25 May 2018 we herewith give you the opportunity to object to the publication of your name, your public website, or both on the website of the Mountain Invasion Research Network (www.mountaininvasions.org). You can object at the time of data submission, but also at any later point of time. If so, please contact the MIREN coordinators (miren.contact@gmail.com) and your personal information will be removed.