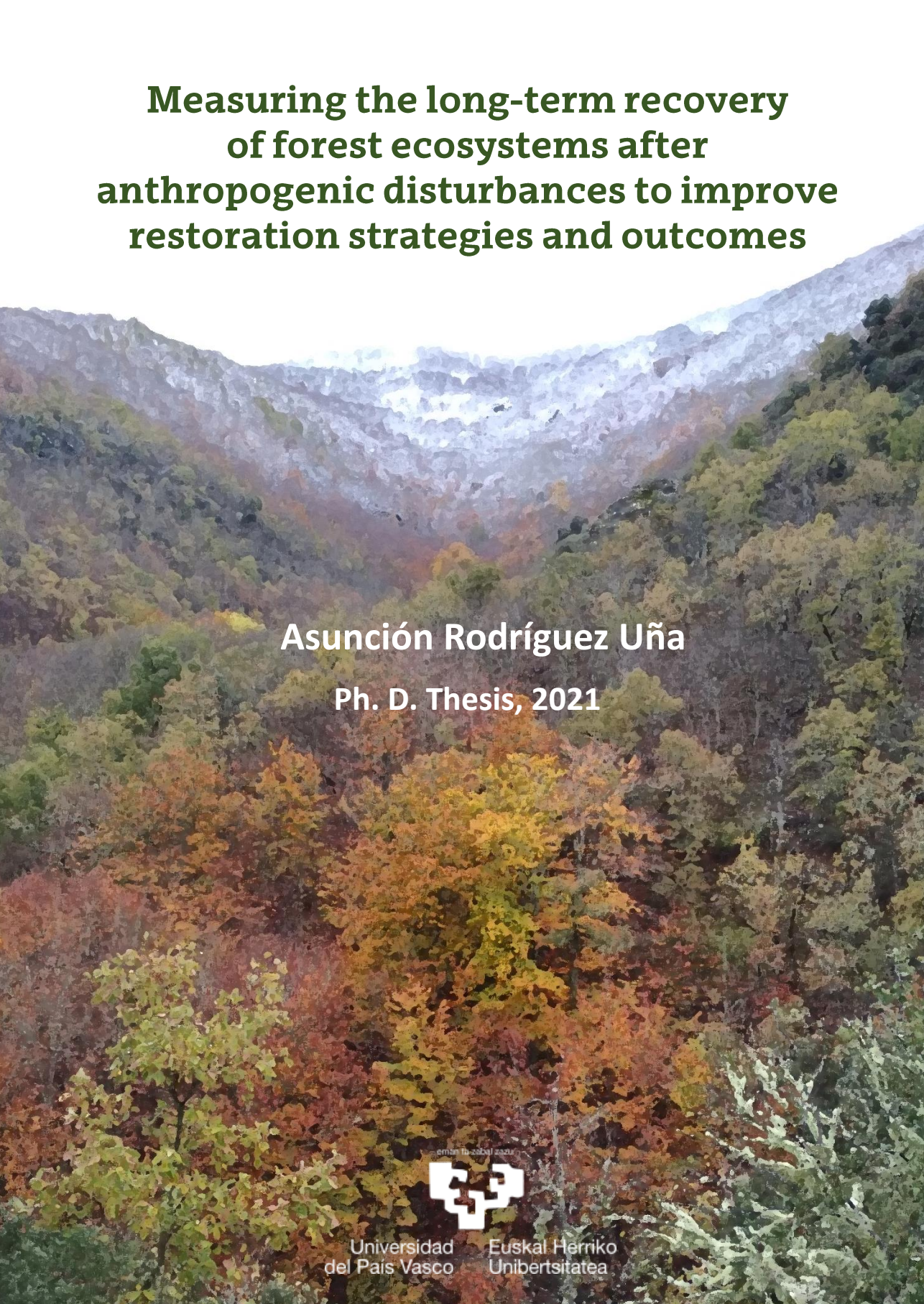
Measuring the long-term recovery of forest ecosystems after anthropogenic disturbances to improve restoration strategies and outcomes



Asunción Rodríguez Uña

Ph. D. Thesis, 2021

eman ta zabal zazu

Universidad del País Vasco

Euskal Herriko Unibertsitatea

Measuring the long-term recovery of forest ecosystems after anthropogenic disturbances to improve restoration strategies and outcomes

Ph. D. Thesis

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April 2021

Thesis supervisors

Prof. David Moreno Mateos

Prof. Isabel Salcedo Larralde

BASQUE CENTRE FOR CLIMATE CHANGE
Klima Aldaketa Ikergai
Sustainability, that's it!

eman ta zabal zazu
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Asunción Rodríguez Uña was funded by the Environmental Fellowship Program of *Fundación Tatiana Pérez de Guzman el Bueno* (2016, http://fundaciontatianapgb.org/), the *María de Maeztu* excellence accreditation (MDM-2017-0714) from the Spanish Ministry of Economy, Industry and Competitiveness and the Basque Centre for Climate Change–BC3.

The research was financed by the National Programme for Research Aimed at the Challenges of Society (CGL2015-70452-R) from the Spanish Ministry of Economy and Competitiveness and the *María de Maeztu* excellence accreditation (MDM-2017-0714) from the Spanish Ministry of Economy, Industry and Competitiveness.

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Thesis images: David Moreno Mateos, June Hidalgo Castañeda and Asunción Rodríguez Uña


A mi familia,
a Carlos y
a este maravilloso planeta,
por hacerme feliz

Agradecimientos

"En la naturaleza nada existe de forma solitaria."
Rachel Carson, Primavera Silenciosa (1962)

Este es para mí sin duda el más especial y esencial momento para culminar esta etapa tan importante de mi vida. Me siento muy afortunada y agradecida a todas esas personas sin las que esta tesis no existiría y estaba deseando poder dedicaros estas palabras de agradecimiento.

La verdad es que yo decidí meterme en este embolado de la investigación sin tener ni idea de lo que era este mundo. Se me presentó como una oportunidad única para poder trabajar en el mundo de la restauración ecológica, que tanto me apasiona, y porque, aunque sé que suena muy de empollona (que lo soy bastante), porque siempre he dicho que soy como una esponja a la que le encanta seguir absorbiendo conocimientos de nuestro maravilloso planeta. ¡Lo que no me imaginaba es que después de estos años la investigación me encantaría y me engancharía tanto! Pero claro, este amor por la ciencia y la restauración no se habrían alimentado en vez de apagado, después de tantos momentos difíciles, si no fuera porque he tenido a mi lado a grandes personas. Personas que me han ayudado a nivel profesional y personal.

Primero apareció la gente del súper departamento de ecología de la Universidad de Alcalá, ¡con ese ambiente tan bueno y único que tienen! Allí me enamoré del trabajo de campo y disfruté muchísimo de ayudar a que personas increíbles realizaran sus investigaciones. Me gustaría agradecer a Pedro y a José María el darme la primera oportunidad de entrar en el mundo científico y permitirme conocer el proyecto en el que he realizado esta tesis. También a Lorenzo por contagiarme su gran pasión por la investigación. A los tres por enseñarme tanto en tan poco tiempo y por animarme a realizar la tesis doctoral. A Loreto, Vero, y Zoë porque el artículo que hicimos las cuatro juntas va a ser al que más cariño voy a guardar y estoy muy orgullosa del trabajo que hicimos y de

nuestras ideas “frescas” ;) Especialmente a Loreto y Vero quiero agradeceros el ayudarme siempre, incluso antes de empezar esta tesis y por haberos convertido en grandes amigas. Loreto, disfruté tanto trabajando contigo que eres la gran culpable de que me animara a hacer la tesis. Compartir experiencias comunes estos años ha sido un gran impulso para mí. Vero, a ti te debo demasiado en esta tesis y necesitarías unos agradecimientos propios jaja ¡mil gracias por tu tiempo y por compartir conmigo tantos conocimientos! También quiero agradecer a Enrique sus sabios consejos, y a Elena su manera tan única de vivir la vida.

Después, me animé, me lié la manta a la cabeza y me marché a Bilbao a hacer la tesis doctoral. Desde entonces mis directores han sido piezas esenciales en este camino de aprendizaje profesional y personal. Quiero agradeceros todo lo que me habéis enseñado, que me ha ayudado a ganar independencia, y también vuestros ánimos en todo momento (sobre todo en este tramo final con tantas curvas). Isabel, gracias por ser una fuente infinita de saber sobre nuestras queridas “micos” y, sobre todo, por estar siempre siempre disponible para ayudarme. David, gracias por confiar en mí y por ver en mí capacidades y cualidades que yo desconocía. Como no, gracias también por el refuerzo positivo constante y ese “tranquila Asun, que lo estás haciendo muy bien” que has repetido infinitas veces y que tanto me hacía falta (espero que cada vez menos).

Muchas gracias a la Fundación Tatiana por darme esta gran oportunidad. Muchas gracias a BC3 por su apoyo económico y a todas las personas de este gran centro con las que he compartido estos años. A June, uno de los mejores regalos que me ha dado esta tesis. Gracias por ser todo comprensión, optimismo y paz. Miles de gracias a los “No tan juniors” por los desahogos en las comidas y cafés, el poteo por Bilbao y tantos buenos momentos que me hacían desconectar y disfrutar. Gracias a especialmente a Mari, Itxaso, Iratxe, Asma, Elena, y Ale, por vuestra amistad y porque con vosotros he compartido este camino de la tesis desde el inicio y habéis sido un gran apoyo. A Teresa, gracias por valorar tanto mis humildes conocimientos y por transmitirme tanta alegría.

Gracias también a Silvestre y Nacho, por tan buenas charletas y ratos de fiesta.

También, quiero agradecer la colaboración crucial de otros investigadores. Alfredo, gracias por acompañarme desde antes de que todo esto empezara y por enseñarme enfoques tan necesarios para la restauración forestal. Muchas gracias a Susana, por tu labor esencial en el estudio de las "micos" y por tus ánimos; a Dani, por compartir conmigo tu tiempo y tus ideas tan interesantes (espero seguir trabajando y aprendiendo contigo); a Antton, por acogerme en Copenhague y enseñarme cómo bordar las PCRs; y a Ibone y Miren por prestarme vuestro laboratorio. Gracias a Antton Gamio por ser una fuente de sabiduría de Artikutza, ese lugar tan mágico y que llevaré siempre conmigo.

Por último, quiero centrarme en mi gente fuera de este mundillo, mis pilares y apoyos externos cuando me entraban ganas de mandarlo todo al fresco. Gracias a mis amig@s incondicionales de Madrid, por tantos años de amistad que nos unen, especialmente a Paloma, Marta y Cris. A pesar de la distancia noto cada día vuestro apoyo y me siento muy afortunada de teneros y saber que siempre vais a estar ahí. A Saioa, por ser otra pieza fundamental, que me ayuda a recodar lo que de verdad importa en la vida. A Isa y Ángel, mis papis adoptivos que me acogieron como nadie cuando llegué a Bilbao. A los "Restauradores", aunque pase el tiempo, os sigo guardando el mismo cariño. Finalmente, quiero darles infinitas gracias a mi familia y a Carlos, los pilares fundamentales de mi vida, antes, durante y después de la tesis. A mis abuelos, especialmente a mi abuelo Miguel, que siempre ha apoyado y creído en mi vocación profesional. A mis padres, Ignacio y Asun, por vuestro amor incondicional, por respetar y apoyar mis decisiones y por conocerme como nadie y saber años atrás que mi camino era la investigación. A mis hermanos, Ignacio y Jesús, por ser un referente para mí de buenos hombres, hermanos y padres, y a Virginia y Nuria por aportar dones únicos a nuestra familia. A Ignacio quiero agradecerle también su experiencia y consejos como investigador. A mis sobrinos, Marcos, Pablo y Jaime, sois el mejor regalo que he tenido en estos últimos años y me habéis llenado de alegría y felicidad cuando más lo necesitaba. A mi

hermana Ana, mi otra mitad, la persona a la que más admiro y la que más me admira. Estos años separadas nos han unido más que nunca y me encanta ver la mujer tan madura y valiente en la que te has convertido, que siempre sabe lo que decirme para animarme, calmarme y apoyarme (gracias también por aportar tu buen gusto a la estética de la tesis ☺). Carlos, millones de gracias por creer en mí y quererme, por compartir tu proyecto de vida conmigo, por seguir a mi lado a pesar de las dificultades y por tu paciencia, apoyo y ayuda todos estos años, ¡y gracias por aguantarme y cuidarme tan bien estos últimos meses! Pero, sobre todo, gracias por hacerme mejor persona y más feliz cada día.

Esta etapa ha sido difícil pero preciosa, y siento que ha merecido muchísimo la pena, porque me ha regalado personas y experiencias únicas e inolvidables. No sé si algún día seré una buena investigadora y, lo más importante, una buena persona, pero desde luego gracias a todos vosotros ahora me siento más capaz, valiosa, segura y orgullosa de mí misma, y para mí ese es el mejor aprendizaje. ¡Millones de gracias!

Acknowledgements

Thanks to June Hidalgo for her help with field work, sample and data processing; to Susana Rodríguez-Echeverría for her collaboration in the study of ectomychorrizal fungi, to Daniel Montoya for his help in the interaction network analysis; to Antton Alberdi for helping with the metabarcoding analysis; to Javier Arbea for the Collembola taxonomic identification; to Julio Cabero for the hypogeous fungi sampling and identification; to Juan Manuel Rubiales for helping with the dendrochronological analysis; to José A. López-López for his statistical collaboration; to Ibone Ametzaga and Miren Onaindia for their support with laboratory space; and to Antton Gamio and the Biodiversity and Environmental Quality Section of San Sebastián City Council for their field work support.

Contents

Chlorociboria aeruginascens

Abstract

Every ecosystem on Earth is now directly or indirectly affected by human activities, impairing biodiversity and key functions and services that ecosystems provide to societies. In order to revert this situation, the science and practice of ecological restoration has become a global priority, especially for forest ecosystems. However, forest restoration might be insufficient to offset the loss caused by anthropogenic disturbances, as restored forests do not commonly recover pre-disturbance levels of structure, functions or services. One reason of this limited success may be that traditional restoration guidelines are based on the short-term recovery of simple ecosystem attributes (e.g. species richness or diversity), or single functions (e.g. soil carbon content). These metrics do not necessarily represent attributes less resilient whose changes operate at larger ecological timescales. Therefore, studies of the recovery of more complex metrics over the long-term are essential to understand forest recovery process and to critically appraise forest restoration outcomes and improve their success.

The main objective of this Ph. D. thesis is to assess the long-term (>100 years) recovery of forests after anthropogenic damages by using metrics with different levels of ecological information. Those metrics that better incorporate ecosystem complexity may be better estimators of the real time required to reach pre-disturbance conditions and could help to improve restoration success. This thesis includes a general introduction (Chapter 1), followed by an opinion chapter (Chapter 2), that contextualizes the investigations developed in chapters 3, 4 and 5. These three chapters explore the forest recovery process along large timescales through both global (Chapter 3) and local (chapters 4 and 5) perspectives. Chapter 6 integrates the principal outcomes of the research chapters and their contribution to improve restoration strategies and outcomes. Finally, Chapter 7 presents the main findings of the thesis.

Chapter 2 addresses four main limitations in forest restoration that compromise its success. We first recommended to take measures during the active land use phase to deal with the common lack of efforts to anticipate and plan restoration. Second, we suggested using multiple references in restoration planning to avoid simplified reference characterization. Third, we advised assessing ecosystem recovery with indicators that incorporate more ecosystem complexity than those traditionally used. Finally, we proposed initiatives to diminish the widespread gap between restoration scientists and practitioners. The following chapters study in detail the approach proposed for the third limitation.

In Chapter 3, we analysed the global forest recovery process and its drivers in the long-term (*c*. 300 years) and estimated their recovery time. We conducted a meta-analysis of 104 forest chronosequences worldwide reconstructing global long-term recovery trajectories of restored forests for six recovery metrics (organism abundance, species diversity, species similarity, carbon cycling, nitrogen stock and phosphorus stock), following agriculture or logging. Our results showed that forest recovery increased over time, with faster recovery following agriculture than logging, especially for abundance, carbon cycling and nitrogen stock, and faster recovery for abundance compared to diversity. Woody plant and invertebrate abundance and similarity and woody & non-woody plant and microorganism abundance reached higher recovery levels than non-woody plants. Our outcomes also suggest that forests worldwide may need over four centuries to recover most of their biodiversity and functions.

Chapters 4 and 5 studied the recovery of below-ground interactions in two open-cast iron mines in northern Spain, in use since the 14th century and abandoned over 107 and 148 years. To assess ecosystem recovery, we used metrics incorporating more complexity (i.e. biotic interactions) than the simplified attributes traditionally studied (i.e. species richness and diversity). Thus, Chapter 4 characterized the interactions between ectomycorrhizal fungi and European beech (*Fagus sylvatica* L.) trees using metrics with distinct levels of ecological information. Species

richness, species diversity and taxonomic diversity of ectomycorrhizal fungi recovered to undisturbed values, whereas species identity was still different. *Basidiomycota* and *Ascomycota* abundances and certain fungal functional traits (i.e. exploration and sporocarp types) also reached undisturbed values. This signal of old mining operations may be partially caused by their long-term impact on soil chemistry, as the differences in soil pH and NH_4 showed an effect on the communities of beech associated ectomycorrhizal fungi. Chapter 5 analysed the recovery of Collembola-fungi interaction networks in the same mines. We investigated the dissimilarities in the structure of Collembola communities and fungal communities present in their gut contents inside and outside the mines. We also evaluate the recovery of several metrics related to their network architecture. Our results show that while Collembola species richness and diversity have recovered inside the mines, their species composition remains different from the preserved surroundings. As with ectomycorrhizal fungi communities, this may be partly due to the differences in soil pH, derived from mining legacies. Collembola-associated fungal communities inside and outside the mines differed in their functional groups, being mutualistic and saprotrophic interactions more frequent than parasitic interactions outside the mines. Networks from undisturbed forests presented unique species and compartmentalized and weakly connected architectures that made them more robust to extinctions than networks within the mines.

Overall, the outcomes of this thesis advance our knowledge about long-term forest recovery and evidenced that forest restoration is a centennial process. The assessment of forest restoration success should be based on the re-establishment of the abiotic conditions and the diversity, composition and interactions of above- and below-ground communities to incorporate the assemblage of ecosystem complexity. Given the long time frames to recover these attributes, the current global restoration strategies should be planned following centennial timescales to set a more feasible scenario to truly restore forests.

Resumen

En la actualidad, todos los ecosistemas de nuestro planeta se han visto afectados, directa o indirectamente, por las actividades humanas, alterando la biodiversidad y servicios y funciones claves que los ecosistemas proveen a la sociedad. Con el fin de revertir esta situación, la ciencia y aplicación de la restauración ecológica se han convertido en una prioridad a escala mundial, especialmente en los ecosistemas forestales. Sin embargo, la restauración forestal puede no ser suficiente para compensar las pérdidas causadas por perturbaciones antrópicas, ya que los bosques restaurados no suelen alcanzar los niveles de estructura, funciones y servicios previos a la perturbación. Este limitado éxito puede deberse a que las directrices de restauración tradicionales se limitan a la recuperación a corto plazo de atributos del ecosistema demasiado simples (p. ej. la riqueza de especies o la diversidad) o sólo una única función (p. ej. contenido de carbono en el suelo). Estas métricas no necesariamente representan atributos menos resilientes cuyos cambios suceden a escalas de tiempo ecológicas más largas. Por tanto, es esencial estudiar la recuperación a largo plazo de métricas más complejas para poder entender el proceso de recuperación de los bosques y evaluar de forma crítica los resultados de la restauración forestal, así como mejorar su éxito.

El objetivo principal de esta tesis doctoral es evaluar la recuperación a largo plazo (>100 años) de los bosques afectados por perturbaciones antrópicas, mediante el uso de métricas con diferentes niveles de información ecológica. Es probable que aquellas métricas que incorporan una mayor complejidad del ecosistema sean mejores estimadores del tiempo que realmente se requiere para alcanzar las condiciones previas a la perturbación y podrían mejorar el éxito de la restauración. Esta tesis incluye una introducción (Capítulo 1), seguida de un capítulo de opinión (Capítulo 2), donde se ponen en contexto las investigaciones desarrolladas en los capítulos 3, 4 y 5. Estos tres capítulos exploran el proceso de recuperación de los bosques a largo plazo mediante una perspectiva tanto

global (Capítulo 3) como local (capítulos 4 y 5). En el capítulo 6 se integran los resultados principales de los capítulos de investigación y su contribución para la mejora de las estrategias de restauración y sus resultados. Finalmente, el capítulo 7 recoge los principales hallazgos de esta tesis.

El Capítulo 2 aborda cuatro limitaciones principales de la restauración forestal que pueden comprometer su éxito. En primer lugar, se recomienda llevar a cabo medidas durante la fase de explotación para lidiar con la escasez común de esfuerzos para anticipar y planificar la restauración. En segundo lugar, sugerimos el uso de múltiples referentes en la planificación de la restauración para evitar una caracterización simplificada del sistema de referencia. En tercer lugar, se aconseja evaluar la recuperación de los ecosistemas con indicadores que incorporen una mayor complejidad de los tradicionalmente utilizados. Por último, proponemos diversas iniciativas para acortar las distancias existentes entre la ciencia de la restauración y su aplicación. Los siguientes capítulos estudian en detalle el enfoque propuesto para la tercera limitación.

En el Capítulo 3 se analiza el proceso de recuperación forestal a escala global y los factores que lo determinan a largo plazo (c. 300 años), así como el tiempo requerido para su recuperación. Se llevó a cabo un meta-análisis de 104 cronosecuencias de todo el mundo para poder reconstruir trayectorias globales de recuperación a largo plazo de bosques restaurados, que incluían seis métricas de recuperación (abundancia de organismos, diversidad de especies, similaridad de especies, ciclo de carbono y stock de nitrógeno y fósforo), tras el impacto de la agricultura y las explotaciones forestales. Nuestros resultados muestran que la recuperación de los bosques se incrementa a lo largo del tiempo, siendo más rápida tras la agricultura que tras la explotación forestal (especialmente para la abundancia de organismos, el ciclo de carbono y el stock de nitrógeno), y más rápida para la abundancia de organismos que para la diversidad de especies. La abundancia y similaridad de plantas leñosas e invertebrados alcanzó niveles de recuperación más altos que la de plantas no leñosas. Nuestros resultados también sugieren que a escala

global los bosques pueden necesitar más de cuatro siglos para recuperar la mayor parte de su biodiversidad y de sus funciones.

*Los capítulos 4 y 5 estudian la recuperación de las interacciones hipogeas en dos minas de hierro a cielo abierto en el norte de España, que estuvieron activas desde el siglo XIV y abandonadas durante más de 107 y 148 años. Para evaluar la recuperación del ecosistema, se utilizaron métricas que incorporan una mayor complejidad, como las interacciones bióticas, que los atributos simplificados utilizados tradicionalmente, como la riqueza de especies o la diversidad. Por ello, en el Capítulo 4 se caracterizaron las interacciones entre los hongos ectomicorrícicos y el haya europea (*Fagus sylvatica *L.), por medio de métricas con distintos niveles de información ecológica. La riqueza y diversidad de especies y la diversidad taxonómica de hongos ectomicorrícicos han recuperado los valores de la zona no perturbada, mientras que la identidad de las especies sigue siendo diferente. Las abundancias de Basidiomycota y Ascomycota y de ciertos rasgos funcionales fúngicos, es decir, el tipo de exploración y de esporocarpo, también han alcanzado los valores encontrados en la zona no perturbada. La señal de las antiguas explotaciones mineras podría estar causada parcialmente por su impacto a largo plazo en la química del suelo, ya que nuestros resultados sugieren que las diferencias en el pH y el NH_4 del suelo han influido en las comunidades de hongos ectomicorrícicos asociados al haya. El capítulo 5 analiza la recuperación de las interacciones colémbolo-hongo en las mismas minas. Para ello, se investigaron las disimilaridades dentro y fuera de las minas en la estructura de las comunidades de colémbolos y de hongos presentes en su contenido gástrico. Nuestros resultados muestran una recuperación de las riqueza y diversidad de especies de colémbolos en las minas, mientras que la composición de especies se mantiene diferente a la de los alrededores conservados. Como en el caso de las comunidades de hongos ectomicorrícicos, esto puede deberse en parte al legado de la minería, que ha dado lugar a un elevado pH del suelo. Las comunidades de hongos asociados a los colémbolos también difieren en sus grupos funcionales, ya que las interacciones mutualistas y saprobias fueron más frecuentes que las parásitas en el exterior de las minas. Las redes de bosques no*

perturbados presentan especies únicas y arquitecturas compartimentadas y débilmente conectadas, favoreciendo una mayor robustez a extinciones que las redes del interior de las minas.

A nivel general, los resultados de esta tesis aumentan nuestro conocimiento sobre la recuperación forestal a largo plazo y evidencian que la recuperación de los bosques es un proceso centenario. Por tanto, la evaluación del éxito de la restauración debería basarse en el restablecimiento de las condiciones abióticas y de la diversidad, composición e interacciones de las comunidades epigeas e hipogeas, para poder así incorporar el ensamblaje de la complejidad del ecosistema. Dado que estos atributos se recuperan en extensos periodos de tiempo, las estrategias actuales de restauración deberían planificarse siguiendo escalas de tiempo centenarias para establecer escenarios más factibles que de verdad permitan restaurar los bosques.

Chapter 1
General introduction

"Here is the means to end the great extinction spasm. The next century will, I believe, be the era of restoration in ecology."

(Edward O. Wilson, 1992)

1.1. Ecological restoration as a global priority

Humans have altered ecosystems for millennia, but the rate, magnitude and extent of anthropogenic impacts in the recent decades are unprecedented. Every ecosystem on Earth is now directly or indirectly affected by human activities, impairing biodiversity and key functions and services that ecosystems provide to societies (Haddad et al., 2015; Newbold et al., 2015; Song et al., 2018). In order to revert this situation, the science and practice of ecological restoration has become a global priority (Gann et al., 2019; Strassburg et al., 2020).

Ecological restoration is generally defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (SER, 2004). It aims to re-establish a self-sustaining and resilient ecosystem to its historic ecological trajectory, including both the organisms and the features and dynamic processes that support them (Balaguer et al., 2014; Holl, 2020; Palmer et al., 2016). The idea of healing damaged lands dates back centuries, but modern ecological restoration began in the 1930s when the renowned conservationist Aldo Leopold started promoting the movement by restoring prairie vegetation and planting pines to reduce soil erosion in Wisconsin in 1935 (Palmer et al., 2016; Van Andel & Aronson, 2012). This discipline gained more relevance in the second half of the 20th century, when international related societies and journals were founded. The Society for Ecological Restoration was established in 1988 and its journals Ecological Restoration and Restoration Ecology were established in 1981 and 1993, respectively. It has since grown, with several international restoration goals being launched in the last decade (Table 1.1), culminating in the declaration of 2021–2030 as the United Nations Decade on Ecosystem Restoration (UNGA, 2019).

Table 1.1. The most important international restoration strategies launched to date, with their specific restoration goals and deadlines.

Year	Initiative	Participation	Restoration goal	By
2021	The UN Decade on Ecosystem Restoration	193 countries	Enhance actions to prevent, halt and reverse ecosystem degradation; increase our understanding of the restoration benefits; and apply this knowledge in education systems and decision-making.	2030
2020	EU Biodiversity strategy for 2030	27 EU member states	To be defined in 2021.	2030
2017	United Nations Strategic Plan for Forests	193 countries	3% increase of forest land through protection, restoration, afforestation and reforestation.	2030
2015	Sustainable Development Goals	193 countries	Restore degraded forests and increase afforestation and reforestation globally.	2020
			Restore degraded land and soil to achieve a land degradation-neutral world.	2030
2014	New York Declaration on Forests	40 countries	Restore 150 million ha of degraded and deforested land.	2020
			Restore at least 200 million ha more	2030
2014	Initiative 20x20	17 countries	Restore 20 million ha of degraded Latin American and Caribbean land	2020
2011	Bonn Challenge	61 countries	Restore 150 million ha of degraded and deforested land.	2020
			Restore 350 million ha of degraded and deforested land.	2030
2011	EU Biodiversity Strategy	28 EU member states	Restore at least 15% of degraded ecosystems.	2020
2010	Aichi Biodiversity Targets	168 countries	Restore at least 15% of degraded ecosystems.	2020

1.2. The special need for forest restoration

Most of the international restoration strategies mentioned above advocate for forest restoration, as forests cover almost 31% of the Earth's land area (4060Mha). They provide essential ecosystem services such as carbon sequestration, erosion control, pollination and nutrient cycling and harbour most of terrestrial biodiversity - containing more than 60,000 tree species and provisioning habitats for 80% of amphibian species, 75% of bird species and 68% of mammal species (FAO & UNEP, 2020). Still, this biodiversity is seriously threatened under current rates of forest loss and degradation (Betts et al., 2017). From 1982 to 2016, there was an overall net gain in tree cover as a result of a net loss in the tropics being outweighed by a net forest afforestation and forest natural expansion in the subtropical, temperate and boreal climate zones (Song et al., 2018). However, deforestation (i.e. permanent forest loss) and forest degradation continue to occur at worrying rates (FAO, 2020). In the last two decades, the world has lost 9.7% of its tree cover (386Mha), equivalent to 105Gt of CO_2 emissions (Hansen et al., 2013). Deforestation is likely the main driver of 27% of global tree cover loss since 2000, with a global rate of deforestation that has remained constant at approximately 5Mha per year (Curtis et al., 2018). Like urbanization (responsible of <1% of tree cover loss), deforestation implies a permanent forest and shrubland loss resulting in nonforest land uses like agriculture or mining. The remaining tree cover loss corresponds to land use changes where forest recovery may begin in subsequent years. It includes forestry operations within managed forests and tree plantations (26%), shifting agriculture (24%), and wildfires (23%) (Figure 1.1; Curtis et al., 2018). However, forest recovery in these areas may be incomplete and the recovery time long, given the currently limited outcomes obtained from forest restoration.

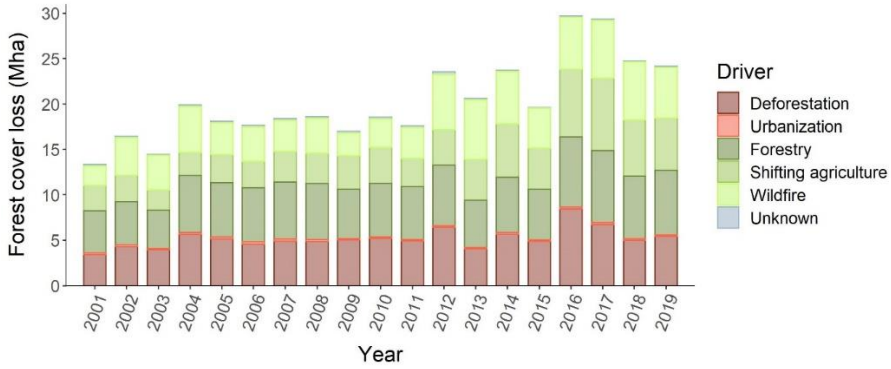

Figure 1.1. Global annual tree cover loss by dominant driver. Reddish drivers imply a permanent conversion from forest and shrubland to a nonforest land use and greenish drivers involve a land use change with likely tree cover regrowth in subsequent years. Data source: The Sustainability Consortium, World Resources Institute, and University of Maryland, "Tree Cover Loss by Driver", accessed through www.globalforestwatch.org.

1.3. Forest restoration limitations

In the last years, several studies have pointed out that forest restoration might be insufficient to offset the loss caused by anthropogenic disturbances, as restored forests may not reach levels of their structure and functions after decades or even centuries (Curran et al., 2014; Jones et al., 2018; Moreno-Mateos et al., 2017). Curran *et al.* (2014) suggested that species richness in restored forests may converge to reference levels within a century, while the recovery of species composition may take up to an order of magnitude longer (hundreds to thousands of years). Similarly, Moreno-Mateos *et al.* (2017) showed that forests do not completely recover the abundance of plants and animals and species diversity or their carbon and nitrogen cycling, after more than two decades since the end of the impact. These studies, and many others reporting forest restoration success (Crouzeilles et al., 2017; Meli et al., 2017; Rey Benayas et al., 2009), support the idea that traditional restoration guidelines are based on the recovery of simple attributes (e.g. species richness or diversity), or single functions (e.g. soil carbon content). This situation may be aggravated by the current prioritisation of forest restoration for global climate regulation (Allen et al., 2019;

Kemppinen et al., 2020), which could increase the risk of focusing restoration efforts on maximizing rates of carbon sequestration while neglecting biodiversity recovery or the recovery of other essential ecosystem services (Lewis et al., 2019).

Forest restoration practices do not commonly consider complex attributes, like below-ground interactions, as the object of recovery (Guerra et al., 2021). This is likely related to the fact that these attributes may take longer to recover than species diversity (Kaiser-Bunbury et al., 2017; Morriën et al., 2017). Studying the recovery of soil biota interactions, such as the mutualistic interaction between plants and mycorrhizal fungi or the trophic interaction between microarthropods and fungi, is crucial as they are key determinants of the restoration of ecosystem structure and functioning (Tedersoo, Bahram, et al., 2020; Wagg et al., 2019). Fungi are essential for organic material decomposition (Tedersoo, Anslan, et al., 2020) and mycorrhizal fungi, in particular, enhance plant water and nutrient access and stress tolerance (Brundrett & Tedersoo, 2018). Soil mesofauna (e.g. Collembola) play a relevant role in nutrient cycling in soils and forming soil microstructure (Chamberlain et al., 2006). These two groups of soil biota are sensitive to soil degradation, making them good indicators of ecosystem recovery (Sterkenburg et al., 2019; Sterzyńska & Skłodowski, 2018).

Another reason that may compromise forest restoration success is our limited understanding of the recovery process at large timescales (>100 years). It is commonplace to monitor direct observation recovery only a few years after the end of the impact, occasionally a few decades, failing to assess the recovery of ecosystem properties that require many decades or centuries (Moreno-Mateos et al., 2020). Therefore, studies of the recovery of more complex ecosystem attributes over the long-term are needed to critically appraise forest restoration outcomes and improve their success (Figure 1.2). As forest degradation proceeds, it is urgent to identify forest restoration limitations and study new approaches to overcome them, particularly, if we aim to achieve those international restoration goals set by 2030.

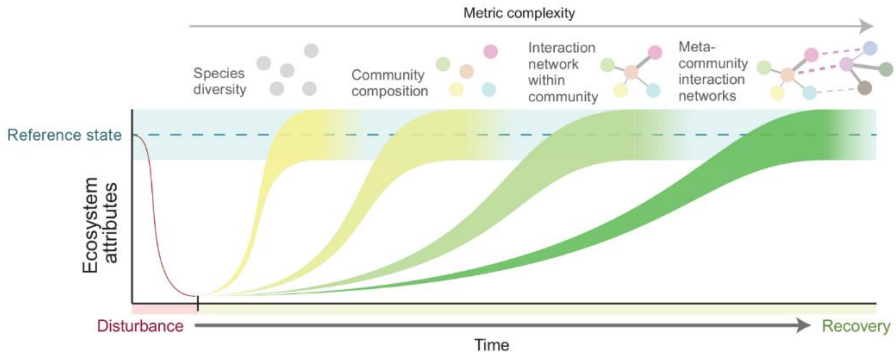

Figure 1.2. Metrics of ecosystem recovery along time. Metrics including more complex ecosystem attributes may require longer recovery periods. Species diversity – species presence and abundance; community composition – identity of species in a community; interaction network within community – metrics based on the links between species; interaction networks between communities – metrics based on the links across multi-layered interaction networks. Source: Moreno-Mateos *et al.* (2020).

1.4. Where to study long-term forest recovery

The study of forest restoration over the long-term requires data from places under recovery for many decades or centuries that have never been degraded afterwards. There is a global scarcity of places fulfilling this criteria, especially in the densely populated temperate regions. The study site of this thesis is the unique beech forest of Artikutza, located in Navarra, northern Spain (Figure 1.3). The presence of iron veins in the soils of Artikutza (Galán et al., 2014), together with its strong slopes, made mining, instead of agriculture or logging, the essential human activity in this place, at least, since the 14th century until the beginning of the 20th century. These mining activities were intrinsically integrated with the deriving iron industry, so they both probably started simultaneously (Mugueta Moreno, 2019). The first documentation about the iron industry in Artikutza dates from 1369, when this place became a property of the *Colegiata de Roncesvalles* and these activities started to be registered (Moreno Mateos et al., 2019).

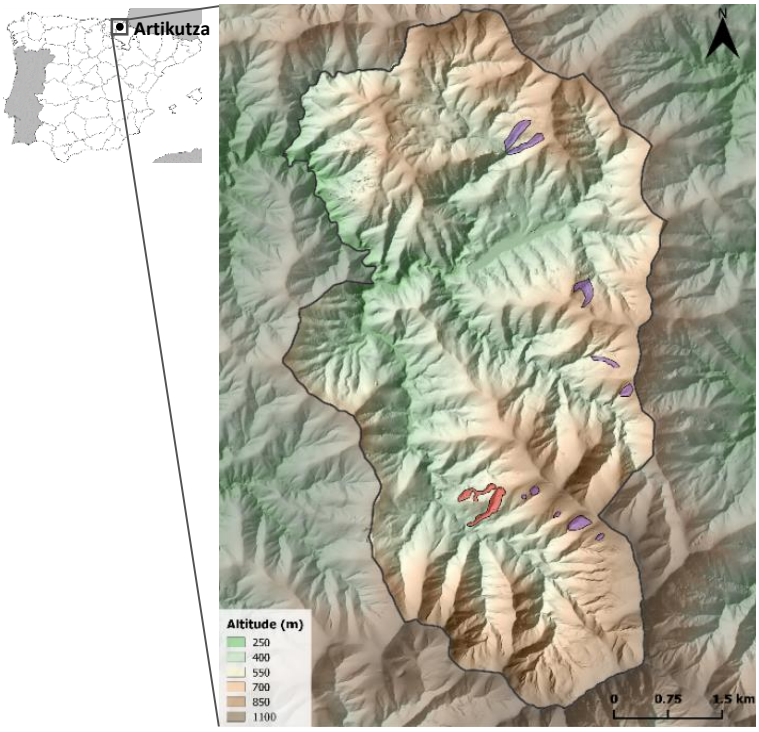

Figure 1.3. Digital elevation model with the location of the open cast iron mines studied in this thesis (in red) and other mines found (in purple) in the beech forest of Artikutza, northern Spain (delimited by the black line). Digital elevation model from *Instituto Geográfico Nacional*, accessed through www.ign.es.

Hence, it is likely that mining operations began earlier but no records have arrived until our days. The poverty of the extracted mineral and the steep topography accelerated the abandonment of the mining activities at the end of the 19th century. The last records of iron extraction date from 1910. In 1919, Artikutza was acquired by the San Sebastián City Council and, since then, no human uses (except recreational activities) were allowed, favouring forest recovery. After more than a century since the cessation of mining activities, numerous remains of this impact can be found along this forest, like remnants of the finery forges used in the iron industry (Figure 1.4a) or deep galleries and cavities (Figure 1.4b). For this thesis we selected the largest open mine exploitation (Figure1.4c), but similar exploitations were found in other areas of Artikutza (Figure 1.3).

In sum, Artikutza is a unique study area because (1) it allows to assess the recovery process from intense impacts; (2) it is currently covered by forests that have remained intact since mining abandonment; (3) it is in the densely populated temperate region; and (4) it covers over a century of recovery.

Figure 1.4. Current signals of ancient open cat iron mining in the beech forest of Artikutza, northern Spain. a) Exterior of the remnants of a finery forge. b) Deep cavities created after iron extraction. c) Views of the largest and oldest mine studied. Source: June Hidalgo Castañeda and David Moreno Mateos.

1.5. Thesis hypothesis and goals

This thesis provides deeper insights into the restoration performance of human-disturbed forests at ecological timescales. *Its main objective is to assess the long-term (>100 years) recovery of forests after anthropogenic damages by using metrics with different levels of ecological information.* We think that those metrics that better incorporate ecosystem

complexity will be better estimators of the real time required to reach pre-disturbance conditions and will help to improve ecological restoration strategies and outcomes. The specific objectives are:

1. To investigate the long-term (*c*. 300 years) recovery process of human-disturbed forests worldwide with traditional recovery metrics related to ecosystem diversity and functions.
2. To assess the recovery of a temperate forest after more than 100 years since the cessation of open cast mining operations by looking at metrics incorporating more complexity (i.e. the below-ground biotic interactions between trees and ectomycorrhizal fungi and between Collembola and soil fungi).
3. To evaluate forest restoration success and time to recovery.

These aims were approached through both global (with a meta-analysis in Chapter 3) and local (with observational studies on an abandoned mine in chapters 4 and 5) perspectives of the forest recovery process along large timescales (Table 1.2).

Exploring biotic interactions could help to identify and favour interactions existing in late stages of ecosystem recovery to accelerate restoration and enhance its success. Estimating the extent to which forests are recovered and the time they potentially need to reach that goal will have direct implications for global restoration initiatives.

1.6. Thesis structure

The thesis includes seven chapters. This general introduction (Chapter 1) is followed by an opinion chapter (Chapter 2), that contextualizes the investigations developed in the next research chapters (chapters 3, 4 and 5; Table 1.2). These three chapters followed a scientific article structure, including an abstract, introduction, material and methods, results, discussion and references. The general discussion (Chapter 6) combines the principal findings of the research chapters and explores their contribution to improve restoration strategies and outcomes.

Finally, the last chapter presents the main conclusions of the thesis (Chapter 7). The central chapters are described in further detail next:

- Chapter 2. *Identifying challenges to current forest ecosystem restoration*. This chapter addresses four main limitations in forest restoration that compromise its success: (1) the absence of measures taken during the active land use phase that would allow to anticipate restoration; (2) the simplified characterization of the reference systems; (3) the short-term assessment of ecosystem recovery using simplified indicators that do not capture ecosystem complexity; and (4) the widespread gap between restoration scientists and practitioners. With colleagues, lead this piece suggesting innovative approaches to overcome these challenges. The following chapters study in detail the approach proposed for the third limitation, to improve the evaluation of restoration performance.

- Chapter 3. *A global evaluation of the long-term recovery of forest ecosystems degraded by anthropogenic disturbances.* This chapter analyses the global forest recovery process and its drivers in the long-term (c. 300 years) and estimates the recovery time. It is a meta-analysis of 104 forest chronosequences worldwide reconstructing global long-term recovery trajectories of restored forests for six recovery metrics (organism abundance, species diversity, species similarity, carbon cycling, nitrogen stock and phosphorus stock), following agriculture or logging. We measured recovery at different moments through time, after at least 50 years since impact cessation, allowing to construct more robust recovery trajectories and more accurately estimate the time that forests need to recover the metrics we were able to use in the meta-analysis.

- Chapter 4. *The long-term recovery of tree-mycorrhizal fungi interactions in European beech forests after mining.* This chapter studies the recovery of a temperate forest in northern Spain after >100 years since the cessation of ancient open cast mining

activities. To assess ecosystem recovery, we used metrics incorporating more complexity (i.e. biotic interactions) than the simplified attributes traditionally studied (i.e. species richness and diversity). Thus, the interactions between ectomycorrhizal fungi and European beech trees were characterized by using metrics with distinct levels of ecological information (i.e. species richness, species diversity, taxonomic diversity and species composition). Results help to better estimate time to recovery and improve our knowledge about the real magnitude of anthropogenic ecosystem degradation.

- Chapter 5. *The long-term recovery of Collembola-soil fungi interaction networks after mining*. This chapter further analyses the long-term recovery of below-ground biotic interactions, by studying Collembola-fungi interaction networks in the same mines than in Chapter 4. We investigate the dissimilarities in the structure of both Collembola communities and Collembola-associated fungal communities inside and outside the mines. We also evaluate the recovery of several metrics related to their network architecture such as connectance, modularity and robustness. Findings will contribute to better understand the mechanisms of network re-assembly after human impacts and to identify which species should be favoured in restoration actions.

Chapters 4 and 5 were developed as part of the research project "*Estimación del tiempo de recuperación de bosques templados tras impactos antropogénicos históricos a lo largo de un gradiente de complejidad*" (REBECOM) from *Ministerio de Economía y Competitividad*. Its main objective was to identify and test metrics of the recovery process of ecosystems degraded by anthropogenic disturbances, to estimate the real magnitude of degradation and determine possible limiting mechanisms of that recovery process that enable to improve future restoration and conservation actions.

Table 1.2. Overview of the research chapters and related publications presented in this thesis.

	Title	Main objective	Recovery metrics	Study area (approach)	Scientific publications
Chapter 3	*A global evaluation of the long-term recovery of forest ecosystems degraded by anthropogenic disturbances*	Measure long-term global forest recovery following agriculture and logging with traditional metrics and estimate time to be completely restored	Ecosystem structure: organism abundance, species diversity and compositio Biogeochemical properties: C cycling and N and P stocks	Worldwide forests (meta-analysis)	Rodríguez-Uña et al. (in preparation to be submitted in *Science*)
Chapter 4	*The long-term recovery of tree-mycorrhizal fungi interactions in European beech forests after mining*	Measure long-term beech forest recovery after ancient mining with more complex metrics: below-ground symbiotic interactions	Beech tree-ectomycorrhizal fungi (EcM) interactions: EcM richness, diversity and composition	Beech forest in northern Spain (observational)	Rodríguez-Uña et al. (under review) *Restoration Ecology* Rodríguez-Uña et al. (2019) *Ecosistemas* Moreno Mateos et al. in *Artikutza. Naturaleza e Historia* (2019)
Chapter 5	*The long-term recovery of Collembola-soil fungi interaction networks after mining*	Measure long-term beech forest recovery after ancient mining: below-ground trophic interaction networks	Collembola-fungi interactions: richness, diversity and composition of both levels, and network architecture	Beech forest in northern Spain (observational)	Rodríguez-Uña et al. (in preparation to be submitted in *Journal of Applied Ecology*)

The research approach used in these chapters (i.e. the study of more complex recovery metrics over the long-term) contributed to set the basis of the scientific publication of Moreno-Mateos et al. (2020) in *Nature Ecology and Evolution*.

Chapter 2
Identifying challenges to current forest ecosystem restoration[1]

[1] Rodríguez-Uña A, Cruz-Alonso V, Rohrer Z, Martínez-Baroja L. 2020. Fresh perspectives for classic forest restoration challenges. *Restoration Ecology* 28: 12-15

Abstract

Restoration ecology is a young scientific discipline with limitations that compromise the recovery of ecosystem biodiversity and functions. Specifically, for forest restoration planning and assessment, we first recommend measures prior to land use changes to deal with the common lack of efforts to anticipate and plan restoration. Second, we suggest using multiple references in restoration planning to avoid simplified reference characterization. Further, we advise assessing ecosystem recovery with indicators that better incorporate ecosystem complexity in recovery assessments. Finally, we propose initiatives to encourage scientific communication outside academia to diminish the communication gap between scientists and practitioners.

Synthesis and applications. Recognizing and solving classic issues in ecological restoration is crucial for this developing science to continue being applicable under the current fast changing environmental conditions. Young restoration scientists' voices may shed fresh views on classical problems and help to overcome future international commitments. Anticipating restoration, using multiple references and indicators that reflect ecosystem complexity, and promoting academia-practitioner partnerships in restoration projects, are feasible approaches already applied by young restoration ecologists to face classic restoration challenges.

Resumen

La restauración ecológica es una disciplina científica joven con ciertas limitaciones que comprometen la recuperación de la biodiversidad y de las funciones de los ecosistemas. Más específicamente, para la planificación y evaluación de la restauración forestal, recomendamos, en primer lugar, la implantación de medidas previas a que se produzcan los cambios de uso de suelo, para poder solventar la habitual falta de esfuerzos para prever y planificar la restauración. En segundo lugar, sugerimos utilizar referentes múltiples en la planificación de la restauración para evitar así una

caracterización simplificada del ecosistema de referencia. Además, aconsejamos evaluar la recuperación de los ecosistemas con indicadores que incorporen mejor la complejidad de los ecosistemas. Por último, proponemos la implantación de iniciativas para fomentar la comunicación científica fuera del ámbito académico, con el fin de disminuir la brecha de comunicación entre científicos y profesionales de la restauración.

Síntesis y aplicaciones. Es crucial reconocer y resolver los problemas clásicos de la restauración ecológica para que esta ciencia en desarrollo pueda seguir aplicándose bajo las actuales circunstancias ambientales tan cambiantes. Las voces de los jóvenes científicos de la restauración pueden aportar nuevos puntos de vista sobre problemas clásicos de la restauración y ayudar a superar los futuros compromisos internacionales. Anticiparse a la restauración, utilizar referentes múltiples e indicadores que reflejen la complejidad del ecosistema, y promover la colaboración entre los científicos y los profesionales de la restauración, son estrategias factibles que ya aplicadas por jóvenes ecólogos para afrontar retos clásicos de la restauración.

2.1. Introduction

Ecological restoration is today a key tool to counteract the global increase of ecosystem degradation and biodiversity loss (Aronson & Alexander, 2013; Bastin et al., 2019), as has been acknowledged by the declaration of 2021-2030 as the United Nations Decade of Ecosystem Restoration (UNGA, 2019). However, restoration ecology is a young discipline, which still faces challenges that need to be urgently addressed (Buisson et al., 2018). Restoration planning and assessment are essential stages of ecological restoration (Figure 2.1).

Both stages are frequently discussed in scientific literature (Higgs et al., 2014), especially in forests (where restoration actions have been traditionally accomplished), showing that they can compromise forest restoration success (Gatica-Saavedra et al., 2017; Vallauri et al., 2005). Here we focus on four challenges for forest restoration planning and assessment which we have encountered as young researchers, and suggest a fresh perspective to overcome these constraints. As young restoration ecologists, we must assume our share of responsibility to the challenges of ecological restoration in a changing world.

2.2. Land use management to anticipate restoration

Planning land use (i.e., exploitation and land use changes) is crucial to reducing degradation of prior uses and costs of subsequent forest restoration (Rey Benayas et al., 2016; Rohrer et al., 2018). Despite having the knowledge and tools to anticipate restoration, planned actions are often not implemented due to ecological and socio-economic reasons. Understanding ecosystems, their components and the interactions between organisms is complex (e.g. seed dispersal, Pesendorfer et al., 2016). Among the socio-economic reasons, for instance there is a traditional misconception that perimetral hedgerows reduce agricultural yields, leading to their elimination in farmlands (Van Vooren et al., 2017).

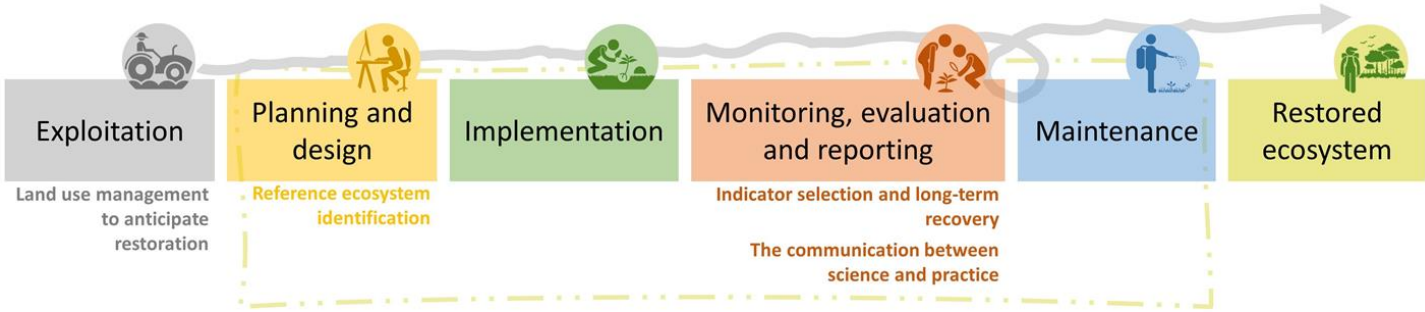

Figure 2.1. Stages of ecological restoration (from the exploitation of resources to the restored ecosystem) based on Gann *et al.*, (2019) (green dotted square). The loop of the arrow represents the adaptive management approach where the monitoring and the maintenance stages repeat as often as necessary to obtain the restored ecosystem. The tips below each stage are discussed in the text.

During the active farming phases, implementing actions, such as planting hedgerows and isolated trees, can maintain seed sources, increase biodiversity, and provide habitats for animals (Manning et al., 2006; Rey Benayas et al., 2008). When degradation is caused by mining operations, actions like maintaining vertical faces can promote rocky habitats for rupicolous vegetation. Incorporating restoration actions during the exploitation may help to accelerate colonization and succession, and maintain rupicolous plant species (Rohrer et al., 2020). These actions may improve ecosystem services during the active land use phase (e.g. pest control) and could shorten the time needed for the ecosystem to recover after land use change (e.g. seed dispersal), reducing restoration costs and catalysing restoration process (Andivia et al., 2017; Pesendorfer et al., 2016). Consequently, despite the ecological and economic limitations, the implementation of planned actions could favour restoration success.

2.3. Reference ecosystem identification

The use of references (i.e., models of the ecosystem condition prior to degradation; Gann et al., 2019) for designing the restoration of degraded sites may be controversial (Aronson et al., 2017; Dufour & Piégay, 2009). First, the inherent uniqueness of each reference and degraded site (i.e., its biotic and abiotic legacies) implies that no reference is a perfect match for a given degraded site (White & Walker, 1997). Moreover, the characterization of references can be costly and time consuming in practice, and it is usually simplified (i.e., by using few reference sites; Ruiz-Jaen and Aide, 2005).

The problem of site specificity could be partially solved by using multiple references for each restored site, selected within a range of similar environmental conditions (White & Walker, 1997). Therefore, a range of variation of reference values could be incorporated in restoration design. Cruz-Alonso *et al*. (2019) identified all possible reference forests using the Spanish Forest Inventory - an open access database of gridded forest plots (Alberdi et al., 2016) -, selecting those

closer than 15 km and 200 m in altitude from the restored forest and within the same forest type. The use of already existing public biotic and abiotic databases to characterize different ecosystem attributes (e.g. land cover maps, historical aerial photographs; Ruiz-Benito et al., 2020) may reduce the cost of using multiple references. To date, this systematic information is not available for forests in most parts of the world, but there is a worldwide trend of sharing biodiversity data (García, 2019). The use of multiple references characterized through biodiversity databases could help restoration design in the near future.

2.4. Indicator selection and long-term recovery

Over the last few years, several studies have concluded that most restored forest ecosystems do not recover the biodiversity and functions that existed prior to disturbance (e.g. Curran et al., 2014; Moreno-Mateos et al., 2017). However, the assessment of ecosystem recovery is usually based on simplified metrics that do not capture the complexity of the ecosystem (e.g. number of species), and that are only measured for a few years after restoration takes place (Montoya et al., 2012; Ruiz-Jaen & Aide, 2005). This may lead to an underestimation of the actual time required for ecosystems to recover.

Long-term monitoring may be unrealistic for practitioners and scientists. A chronosequence-based approach (i.e., space-for-time substitution) might be a feasible alternative for scientists to explore ecosystem recovery in the long-term (Cruz-Alonso et al., 2019; Sutherland et al., 2016). This method requires the disturbance cessation date, but records are often not available, thus, using dating techniques becomes necessary. For example, to date the abandonment of ancient mines later covered by a beech forest, several techniques can be used (1) consulting local history records; (2) using optically stimulated luminescence to determine when sediments rich in silica were last exposed to sun radiation; (3) using dendrochronological approaches to estimate the beginning of tree recruitment after perturbation.

Alternative approaches with metrics integrating higher complexity (e.g. interaction networks and multifunctionality indexes (Cruz-Alonso et al., 2019; Rodríguez-Uña et al., 2019) and for longer time periods should be applied, as many ecosystems may need centuries or more to fully recover (Curran et al., 2014; Rey Benayas et al., 2015).

2.5. The communication between science and practice

Restoration ecologists develop new knowledge, but what is studied is not always focused on what needs to be developed in practice, and scientists frequently lack experience or opportunities to carry out applied programmes (Hopkinson et al., 2017). Furthermore, they do not have easy access to knowledge produced outside academia. On the other hand, most practitioners have limited access to scientific information, such as scientific journals (Amano et al., 2016). Moreover, their perspectives are not always aligned with the theoretical models for ecological restoration (Aronson et al., 2017) and finally, business-as-usual approaches, such as monospecific and linear revegetation, seem hard to abandon.

To overcome these issues, effective academia-practitioner collaboration and sharing information is essential (Meli et al., 2019). Collaborations would enable scientists to test their knowledge (Castillo, 2000) and help practitioners to improve practices toward making scientific based decisions. For example, through an innovative partnership with the University of Castilla-La Mancha, the mining company LafargeHolcim Spain is restoring natural vegetation at a quarry, based on the university's studies on secondary succession (Usarek et al., 2018). The mining company has partnerships with other organizations as well, such as non-profit NGOs like the FIRE Foundation (Rohrer, 2019). Furthermore, scientists could provide the knowledge to Administrations so they can include ecological requirements in their restoration programs, thus encouraging practitioners to prioritize these practices in their project planning and design.

Finally, in the current competitive and publishing driven environment in science (Mazón et al., 2019), scientists should be rewarded for communicating their findings outside of academia (Castillo, 2000), e.g. when applying for official scholar quality assessments and accreditations such as the ANECA in Spain (ANECA, 2019), or when applying for project funding. Efforts towards finding common working grounds and sharing knowledge is crucial to achieving ecological restoration goals.

2.6. Conclusions

In the coming decades, young restoration ecologists will continue to face old problems in a rapidly changing environment, and they will play an essential role in developing ecological restoration as a useful tool to revert ecosystem degradation. Here we propose fresh approaches to overcome some of the main challenges in forest restoration planning and assessment. First, we call for planning measures prior to land use changes to facilitate restoration. Then, we recommend the use of multiple references when planning ecological restoration and the use of indicators integrating forest complexity to assess long-term recovery. Finally, we address how to reduce the science–practice communication gap by promoting knowledge dissemination outside academia. All of these proposals could be integrated to better accomplish international forest restoration initiatives.

Chapter 3
A global evaluation of the long-term recovery of forest ecosystems degraded by anthropogenic disturbances

Abstract

Ecological restoration is today globally implemented to mitigate forest degradation and biodiversity loss. However, restored forests do not commonly recover pre-disturbance levels of structure, functions or services. One obstacle for forest restoration success may be our limited understanding of the recovery process at large timescales (>100 years).

To reconstruct global long-term (*c*. 300 years) recovery trajectories of restored forests, we compiled data from 104 chronosequences worldwide of six recovery metrics (organism abundance, species diversity, species similarity, carbon cycling, nitrogen stock and phosphorus stock), following agriculture or logging.

Our results show that forest recovery increased over time, with 0-4.60 times faster recovery following agriculture than logging, and 0.09-1.06 times faster recovery for abundance compared to diversity. When each recovery metric was analysed individually, we found that abundance, carbon cycling and nitrogen stock recover 0.20–2.37, 0.46–2.30, 0.89–7.18 faster following agriculture than logging, respectively. The abundance of woody plants, woody & non-woody plants, microorganisms and invertebrates reached higher levels of recovery than non-woody plants. The recovery of species similarity of woody plants and invertebrates was also higher than for non-woody plants. Our outcomes also suggest that forests worldwide may need over four centuries to recover most of their biodiversity and functions.

Synthesis and applications. This centennial recovery of forests urges us to plan the current global restoration strategies and environmental regulations accordingly in terms of timescale and environmental impact level.

Resumen

En la actualidad, la restauración ecológica es una práctica mundialmente extendida con el fin de mitigar la degradación de los bosques y la pérdida de biodiversidad. Sin embargo, los ecosistemas restaurados no suelen recuperar los niveles de estructura, funciones y servicios previos a la perturbación. Uno de los obstáculos del éxito de la restauración forestal puede deberse al limitado conocimiento que existe sobre los procesos de recuperación a largo plazo (>100 años).

Para reconstruir las trayectorias de recuperación a largo plazo (de aproximadamente 300 años) de los bosques restaurados, recopilamos datos de 104 cronosecuencias a lo largo de todo el mundo que incluían seis métricas de recuperación (abundancia de organismos, diversidad y similaridad de especies, ciclo de carbono y stock de nitrógeno y de fósforo), tras el impacto de la agricultura y las explotaciones forestales.

Nuestros resultados muestran que la recuperación forestal se incrementa con el tiempo, siendo entre cero y 4,60 veces más rápida tras la agricultura que tras la explotación forestal y entre 0,09 y 1,06 veces más rápida para la abundancia de organismos que para la diversidad de especies. Cuando se analizó cada métrica de recuperación individualmente, se encontró que la abundancia de organismos, el ciclo de carbono y el stock de nitrógeno se recuperan 0,20-2,37, 0,46-2,30, y 0,89-7,18 veces más rápido tras la agricultura que tras la explotación forestal, respectivamente. La abundancia de plantas leñosas, "plantas leñosas y no leñosas", microorganismos e invertebrados alcanzó niveles de recuperación más altos que la de las plantas no leñosas. La recuperación de la similaridad de especies de plantas leñosas e invertebrados también fue mayor que la de plantas no leñosas. Nuestros resultados también sugieren que a escala global los bosques pueden necesitar más de cuatro siglos para recuperar la mayor parte de su biodiversidad y sus funciones.

Síntesis y aplicaciones. Esta recuperación centenaria de los bosques evidencia que las actuales estrategias mundiales de restauración y las regulaciones ambientales deben planificarse de acuerdo a estas escalas de

tiempo y al nivel de impacto ambiental realmente originado.

3.1. Introduction

In our increasingly human-dominated Earth system (Haddad et al., 2015; Song et al., 2018), ecological restoration has become a global priority to revert ecosystem degradation and biodiversity loss (Gann et al., 2019; Strassburg et al., 2020). The global need for restoring damaged ecosystems is now supported by the declaration of 2021–2030 as the United Nations Decade of Ecosystem Restoration (UNGA, 2019). The 'Bonn Challenge' and the New York Declaration on Forests also seek to globally restore 350 million ha of degraded land by 2030 (IUCN, 2017; United Nations, 2014). These strategies mostly focus on the restoration of forests as they harbour 75% of terrestrial biodiversity (FAO, 2016) and provide essential ecosystem services such as erosion control, pollination, nutrient cycling and global climate regulation (Allen et al., 2019; Kemppinen et al., 2020). Recently, >4000Mha have been globally identified for forest restoration to mitigate climate change (Bastin et al., 2019).

Despite the importance of forest ecosystem preservation, most restored forests do not recover to pre-disturbance levels of structure, functions or services (Jones et al., 2018; Moreno-Mateos et al., 2017). One of the reasons that may compromise restoration success is our limited understanding of the recovery patterns of forest ecosystems at a global scale and at ecologically relevant timescales (>100 years) (Hastings, 2016). The lack of studies monitoring recovery using direct observations at those timescales can be partially addressed by the use of space-for-time substitutions to reconstruct recovery trajectories (Cruz-Alonso et al., 2019; Walker et al., 2010). Previous meta-analyses of forest recovery have reported times to recovery ranging from 30 to 180 years, depending on the disturbance type and the recovery metric. They reported longer times to recovery for impacts with strong legacy effects like agriculture and for species richness or diversity rather than for organism abundance or biomass production (Martin et al., 2013;

Meli et al., 2017; Spake et al., 2016). However, they only included the latest condition of the restored ecosystem to construct their recovery trajectories. Here, we built *c*. 300-years composite trajectories of the six most commonly found recovery metrics related to biodiversity and biogeochemical functions, i.e., organism abundance, species diversity, species similarity, carbon cycling, nitrogen stock and phosphorus stock, of forest ecosystems following two of the most widespread impacts, i.e., agriculture and logging. Our chronosequence meta-analysis approach allows us to (1) understand the effect on the recovery process of metrics reflecting different levels of ecosystem complexity and disturbances with contrasting intensity; (2) calculate the recovery completeness of each metric in the long-term and estimate the time needed for each metric to recover; and (3) calculate the rate of change of the recovery process through time.

3.2. Methods

Study selection

We conducted a literature search, on February 2017, in the scientific database ISI Web of Science using the search chain "(chronoseq* and forest* and (recover* or restor* or rehab* or regen*) not fire)". We excluded fire disturbance because all studies in a preliminary search corresponded to natural fires. The search produced 585 results, but 229 were rejected after a title and abstract screening (Figure S1.1 for PRISMA flow diagram (Moher et al., 2010)). From the remaining studies, we selected those that (1) included forest recovery information for at least 50 years; (2) had at least three measurements of recovery in time, including a pre-disturbance reference, an old-growth forest or a mature forest that have been recovering for at least 100 years (hereafter, reference forest); (3) reported time since recovery started; (4) were related to any anthropogenic disturbance (except fire); and (5) included outcome measures of organism abundance, species diversity, similarity or , carbon cycling, nitrogen stock and phosphorus stock. We excluded

less common recovery metrics, mainly related to biogeochemical functions such as soil pH, to reduce database heterogeneity. Our selection yielded 91 published primary studies including 104 chronosequences of recovering forest ecosystems (Figure 3.1; Figure S1.1; see references in Appendix S1).

Database construction

From the selected chronosequences, we extracted 580 outcome measures, i.e. field-based quantitative measurements of ecosystem integrity repeated through time, reported in tables, figures and text of the selected studies. Thus, each outcome measure included several data-points, defined as the value of the ecosystem metric at different times since recovery started (hereafter, recovery time). We used the free software Engauge Digitizer version 11.2 (Mitchell et al., 2017) to extract data from the figures. To increase the homogeneity of the database, prior to data extraction, we carried out an initial training test by the three people entering data. The test consisted in simultaneous data extraction of the same three studies and a posterior agreement on discrepancies. Every two weeks the extracting team met to solve further discrepancies.

Our selection included outcome measures related to agriculture (n = 340, 59%) or logging (n = 240, 41%) disturbances and whose recovery time ranged from 50 years to 295 years (mean ± s.e., 88 ± 4 years; median = 80) (Table S1.1). The outcome measures related to organism abundance (n = 175, 30%) contained measurements of: *(i)* biomass, density and cover of trees, shrubs and herbs, *(ii)* height, diameter, basal area of trees, and *(iii)* biomass and density of bacteria, fungi and invertebrates. The outcome measures related to diversity (n = 148, 28%) included measurements of species richness and diversity indexes, e.g. Shannon and Fisher's alpha. Community composition outcome measures (n = 104, 18%) contained information about species composition at different recovery times along the chronosequence, which were used to calculate pairwise compositional similarity between each data-point and the reference value. We used three common

similarity metrics: Morisita-Horn and Bray-Curtis indices, which account for species relative abundance, and Jaccard index, which considers species occurrence (Legendre & Legendre, 1998). Abundance, diversity and similarity outcome measures included six life forms: woody plants (n = 267), non-woody plants (n = 26), woody and non-woody plants combined (n = 37), invertebrates (n = 65), microorganisms (n = 18), fungi (n = 12) and birds (n = 2). The cycling of carbon included pools (n = 78, 13%) in soil (n = 53), plants (n = 9) and litter (n = 16), and fluxes (n = 13, 2%), i.e. rates of decomposition, respiration or sequestration in soil (n = 8), plants (n = 1) or litter (n = 4). The outcome measures related to nitrogen (n = 45, 8%) and phosphorus (n = 17, 3%) stocks included pools in soil (n = 44), plants (n = 11) and litter (n = 7).

For each outcome measure, we also recorded the following information: latitude and longitude, area of the study site, number of chronosequences in each study, number of data-points along the chronosequence, number of reference sites, number of plots in each data-point and their area, the number of sampling-points (i.e. replicates within plots) and their area, and the restoration strategy (passive (92%) *vs*. active (8%)). Average values were considered across various data-points with the same recovery time (n = 42, in 13 studies). We found data from 7,829 plots, whose size ranged from 320 m^2 to 30 ha. The sizes of the study areas (reported in 90% of cases), ranged from 48 ha to 31,285 km^2.We estimated a total accumulated study area >156,000 km^2 (Table S1.1). With the coordinates of each outcome measure, we extracted mean annual temperature and precipitation from WorldClim 2.1 (http://worldclim.org). We calculated the Lang aridity index (Lang, 1920) by dividing the precipitation by the temperature. Further database construction details are described in Appendix S1.

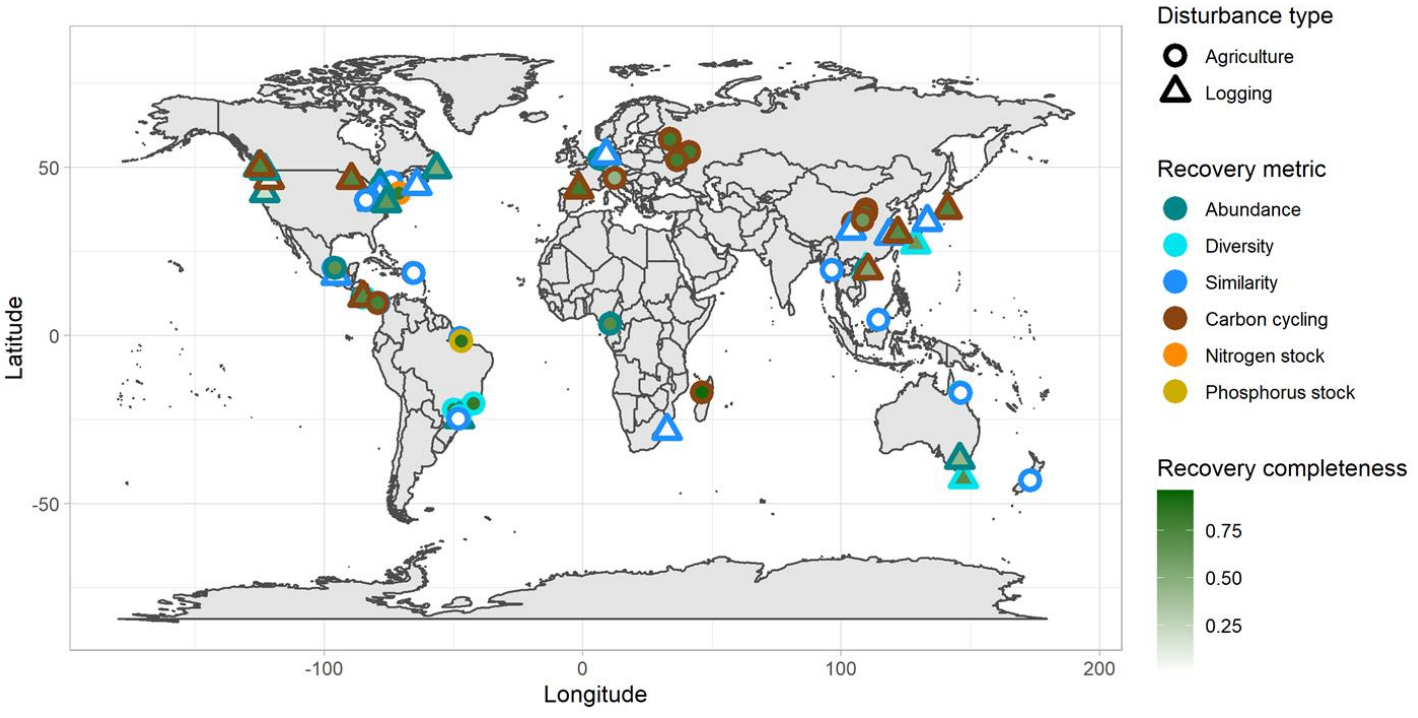

Figure 3.1. Global distribution of the selected chronosequences. It includes our 104 chronosequences with the estimated recovery completeness of the metric with the lowest effect size after 50 years since disturbance cessation. It was calculated on the basis of a model of the response ratio of organism abundance, species diversity, carbon cycling and nitrogen and phosphorus stocks and a model of the effect size of species similarity, following agriculture or logging (see *Methods*).

Effect sizes calculations

We used response ratios to estimate the recovery completeness, i.e. the effect sizes between reference and recovering systems, for abundance, diversity and biogeochemical outcome measures (Koricheva & Gurevitch, 2014; Nakagawa & Santos, 2012). We computed the response ratio for each data-point along the chronosequence as $\ln(\frac{X_{res}}{X_{ref}})$, where X_{res} is the value of the ecosystem metric at a certain recovery time and X_{ref} is the reference value of the same metric in the reference forest. The reference value was from a nearby old-growth forests or the same forest ecosystem in the pre-disturbance state. When none of these options were available (in 33 studies), we used the last available data-point beyond 100 years. In 32% of the cases, recovering values exceeded reference values ($X_{res} > X_{ref}$). This was specially the case in early recovery stages for measurements of plant density, species richness and soil nitrogen concentration.

Metrics from ecosystems recovering from disturbance, such as restored ecosystems, may show higher variability than in pre-disturbance states (Fraterrigo & Rusak, 2008; Murphy & Romanuk, 2012), which commonly provides values of those metrics that go above reference values for extended periods of time (Isbell et al., 2019; Rozendaal et al., 2019). The fact that ecosystem performance values are above the reference do not necessarily involve better, nor worse, ecosystem performance, it involves a deviation from the reference. For this reason, we give those values the same weight than the ones below the reference and consider all together as deviations from the reference. In these cases, we computed the inverted response ratio as $\ln(\frac{X_{ref}}{X_{res}})$ (Marchand et al., 2021; Moreno-Mateos et al., 2017). Response ratios cannot be calculated for outcome measures with zero values; thus, we excluded 56 (2.3%) comparisons where $X_{res} = 0$ (n = 41) or $X_{ref} = 0$ (n = 15). In the case of similarity outcome measures, we estimated the effect size using the value of each similarity index, as these indices already represent a

response metric between reference and recovering systems. We accumulated 2,788 quantitative comparisons between reference and successional recovery stages.

We also estimated the rate of change at each recovery time (t_i) for the abundance, diversity and biogeochemical outcome measures as $\left|\frac{RRt_i - RRt_{i-1}}{t_i - t_{i-1}}\right|$. In the case of similarity outcome measures, we computed the rate of change as $\left|\frac{\text{similarity index } t_i - \text{similarity index } t_{i-1}}{t_i - t_{i-1}}\right|$. We accumulated 2,256 rates of change of the outcome measures through time.

Weighting

Effect sizes in meta-analyses are usually weighted by study precision, which is typically the inverse of the variance. However, as in most ecological meta-analyses (Crouzeilles et al., 2016; Koricheva & Gurevitch, 2014; Rey Benayas et al., 2009), the data necessary to determine variance was absent in the majority (68%) of our outcome measures. Alternatively, we estimated the precision as the product of the number of sampling-points and their area, as a higher sampling effort may imply a higher precision (for further explanation see Appendix S1; Figure S1.2; Figure S1.3). The value of the heterogeneity statistic I^2 index (Higgins & Thompson, 2002) for the response ratio and the similarity effect size was 95.48% (95.43 to 95.54%, 95% confidence interval) and 89.04% (88.64 to 89.43%), respectively, suggesting that among-study heterogeneity (τ^2) accounted for most variation. The random-effects meta-analytic model fitted the data better than the fixed-effect meta-analytic model in terms of the Akaike Information Criterion (AIC) (Burnham & Anderson, 2002). Furthermore, the random-effects model allows generalization of results beyond the sample of integrated studies, as intended in this study (Borenstein et al., 2010). Thus, we assumed random-effects models throughout. For the rate of change, the value of τ^2 was zero, so that fixed- and random-effects weights were equivalent.

Statistical analysis

To analyse the effect on the recovery process of different metrics (i.e. abundance, diversity, carbon cycling and nitrogen and phosphorus stocks) and disturbance types (agriculture vs. logging), we fitted a linear mixed model for the response ratio. We included in the model the triple interaction recovery time × recovery metric × disturbance type, and aridity (Lang aridity index) and restoration strategy were considered as confounding factors (e.g. Cruz-Alonso et al., 2019). As the recovery process along time is unknown, we considered linear and decelerating (logarithmic and square root) recovery time trends, finding logarithmic recovery trend to best fit to the data according to the minimum AIC criterion. The outcome measure identity nested within study was considered as random effects to account for non-independence among response ratios of the same outcome measure or study. Response ratio values ranged from -∞ to 0, so their absolute values were log-transformed to meet the assumptions of general linear models, and then multiplied by -1 to facilitate interpretation. We used a subset of the full dataset including recovery times from 0 to 150 years to guarantee that most metrics were represented along the whole range of recovery time.

To calculate the recovery completeness of each metric, we fitted separate linear models to each recovery metric. Models for abundance, diversity, similarity, carbon cycling and nitrogen and phosphorus stocks included the interaction recovery time × disturbance. Other model specifications were similar to those of the model including all metrics, except for the transformation of similarity values, where the logit-transformation of Bray-Curtis similarity index was used. We used Bray-Curtis index because a preliminary test informed that it was similar to Morisita and Jaccard indices (see Appendix S2; Table S2.3). The models of abundance, diversity and similarity included the life form as a confounding factor. Restoration strategy could not be included in the similarity and phosphorus stock models because there were no cases of active restoration. The best model for abundance, diversity and nitrogen stock followed a logarithmic recovery trend. The best model for carbon cycling and phosphorus stock followed a square root recovery trend and

similarity followed a linear trend. One outlier was removed from nitrogen model, as it was so close to zero that after being log-transformed it resulted in a response ratio of -7.7 (the closest value was -4.7). Using the resulting models, we predicted the percent recovery with respect to reference values (i.e., response ratio or similarity index) at different recovery times: the maximum recovery time found for each metric and 73, 146 and 219 years of recovery (i.e. one, two and three times the global life expectancy (United Nations, 2019)). We further predicted the time needed for forest ecosystems to recover to 90% of reference values. Reaching 100% recovery might be theoretically unfeasible due to decelerating nature of the functions that best explains recovery trajectories, and also ecologically unfeasible due natural variability, the uniqueness of each ecosystem, and the effects of global changes that would prevent an exact match of the reference system (Poorter et al., 2016). For each recovery metric we calculated the median time to recover from a set of recovery times calculated for each level of the categorical variables (i.e., disturbance category, life form or restoration strategy) retained in the best model in each case.

On the basis of the fitted models, we estimated for each of the 104 chronosequences the recovery completeness of the metric with the lowest response ratio after 50 years since impact cessation. We used the model of the response ratio of all recovery metrics together and the model of the effect size of Bray-Curtis similarity index.

To explore the factors behind the rate of changes of the recovery process, we fitted a linear mixed model for the rate of change. We fitted a separate model for the rate of change of Bray-Curtis similarity index (see Appendix S2; Table S2.6). Both models included recovery time and disturbance type as predictor variables, and the first one included the recovery metric as well. The confounding factors were aridity and restoration strategy, and for the similarity model just aridity. We included the outcome measure identity nested within study as random effect. Both models included a logarithmic effect of recovery time according to the same procedure than for the models of the recovery completeness. The rates of change were log-transformed to meet

assumptions of general linear models. We used a subset of the full dataset including recovery times from 0 to 150 years to guarantee that most metrics were represented along the whole range of recovery time.

Models were fitted using R 3.6.3 (R Core Team, 2020) package *lme4* (Bates et al., 2015). In all cases, we standardized continuous explanatory variables by subtracting the mean and dividing by the standard deviation (Schielzeth, 2010). We selected the explanatory variables that better predicted the response for each model using the *dredge* function from *MuMIn* package (Barton, 2020). The best model (i.e. the most plausible to explain a substantial proportion of variance in the data) was the most parsimonious with a $\Delta AIC \leq 2$ in comparison to the minimum AIC among all models (Table S2.1; Table S2.2; Table S2.4 to Table S2.7). We evaluated the good-of-fitness of each model by comparing the observed and the fitted values (Figure S2.1 to Figure S2.11). To avoid non-numeric results after transformations, we removed 46 values equal to 0 or 1 from the effect size database (1.6%) and 53 values equal to zero from the rate of change database (2.3%).

3.2. Results

Forest recovery drivers

We found that forest recovery completeness increased over time, with 0-4.60 times (range referred to the minimum and maximum differences between the 95% confidence intervals of trends compared) faster recovery following agriculture than logging, and 0.09-1.06 times faster recovery for abundance than for diversity outcome measures (Table S3.1; Table S3.2). When separate models were fitted for each recovery metric, we also found an increase in the recovery completeness over time for all of them (Figure 3.2). Abundance, carbon cycling and nitrogen stock recovered 0.20–2.37, 0.46–2.30, 0.89–7.18 times faster following agriculture than logging, respectively (Table S3.3; Table S3.4). The recovery of organism abundance and species similarity (Bray-Curtis index) differed across life forms (Table S3.3). The abundance of woody

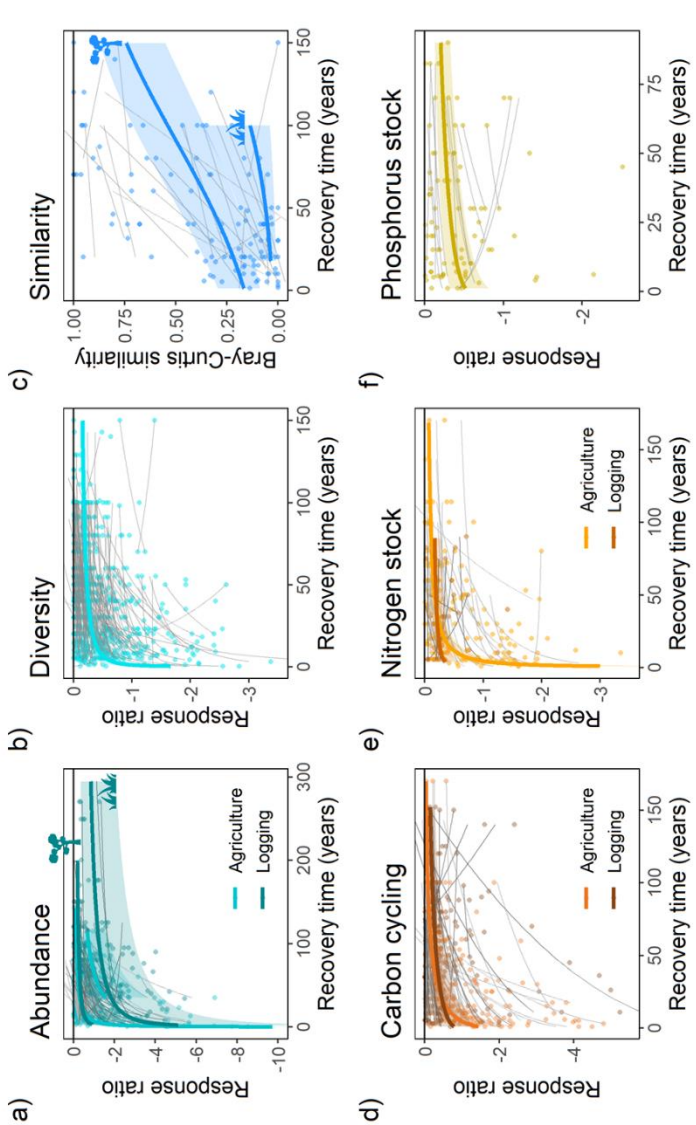

Figure 3.2. Recovery trajectories of forest biodiversity and functions. a) Organism abundance, b) species diversity, c) similarity, d) cycling of carbon, e) nitrogen stock and f) phosphorus stock. Grey lines represent individual chronosequences and dots represent raw measures of the recovery completeness, computed as response ratios (a, b, d-f) or Bray-Curtis similarity effect sizes (c) (see *Methods*). The black horizontal line on y = 0 represents the reference goal value. Thick lines indicate the result of the models fitted for each metric and shadowed areas include the 95% confidence intervals of the fixed effects. Icons designed by Jacqueline Fernandes and BomSymbols (The Noun Project), accessed through www.thenounproject.com.

plants, woody & non-woody plants, invertebrates and microorganisms reached higher recovery levels than non-woody plants. The recovery of species similarity in communities of woody plants and invertebrates was higher than in communities of non-woody plants (Table S3.5; Table S3.6; Figure S3.1). Restoration strategy did not show strong predictive effects on any of the recovery metrics and aridity only showed strong predictive effects on phosphorus stock, whose recovery was negatively affected by aridity (Table S2.4; Table S3.3).

After 50 years since impact cessation, the studied recovering forests were mostly limited by the recovery of carbon cycling (33% of total chronosequences), species similarity (27%) and abundance (26%). Species similarity had the lowest recovery completeness values (all were <0.2 from a value interval ranging from 0 to 1; Figure 3.1; Figure S3.2).

Time to recovery

We could evaluate recovery trajectories spanning from 90 (phosphorus) to 295 years (abundance of non-woody plants following logging). Over those years, recovery reached values ranging from 13.7% (4.1-37.4%, 95% confidence intervals) for similarity of non-woody plants to 97.0% (94.5-98.4%) for carbon recovery in former agricultural areas (Figure 3.3; Table S3.7). The estimated percentage of recovery of each metric after 146 years ranged from 26.6% (8.3–59.3%) for similarity of non-woody plants to 96.00% (92.8–97.8%) for carbon cycling following agriculture. After 219 years, the percentage of recovery ranged from 38.39% (10.2–67.7%) for abundance of non-woody plants following logging to 98.31% (96.7–99.1%) for carbon cycling following agriculture (Figure 3.3; Table S3.8).

The time (median ± s.e.) required for each recovery metric to recover to 90% of reference values was estimated in 127 ± 148 years for organism abundance, 414 years for species diversity, 184 ± 40 years for similarity, 144 ± 60 years for carbon cycling, 417 ± 321 years for nitrogen stock

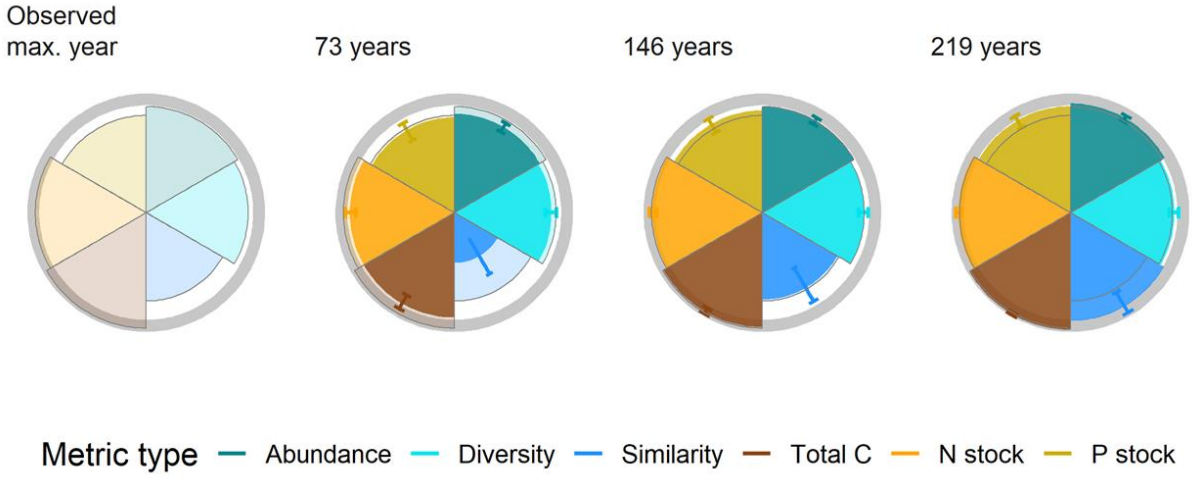

Figure 3.3. Expected recovery status through time: The left light coloured pie chart represents the maximum recovery time found for each metric. The three right dark-coloured pie charts represent the status of the metrics (predicted percent recovery and 95% confidence interval) measured in the selected forests after 73, 146 and 219 years of recovery (i.e. one, two and three times the global life expectancy). The thick grey circle around each pie-chart represents the 90% threshold of reference values.

and 196 years for phosphorus stock (Figure 3.3; Table S3.9). The variability in the time to recovery for diversity and phosphorus stock could not be calculated because they were not affected by the disturbance category, life form or restoration strategy.

Rate of change

The rate of change of the outcome measures decreased through time, but this pattern differed among recovery metrics. The rate of change decreased 0.27-1.57 and 0.31-1.5 times faster for abundance and diversity than carbon, respectively (Figure 3.4; Table S3.10 to Table S3.12). We also found that the rate of change increased with the aridity index (i.e. with lower aridity; Table S3.10).

3.3. Discussion

Our results support and expand in time the hypothesis that the recovery of forests degraded by anthropogenic disturbances is a centennial process (Cole et al., 2014; Curran et al., 2014). We have found that forests globally will likely require beyond 400 years to recover considering that the ecosystem attributes we could gather data for may not be representative of less resilient attributes that better capture the complexity of ecosystems, like belowground interactions and linked functions (Bardgett & Van Der Putten, 2014; Moreno-Mateos et al., 2020). The results also suggest that the impact of logging on ecosystems may be higher than previously thought, given that logged forests recovered more slowly than forests recovering on agricultural grounds. Formerly logged forests needed more time to recover the abundance of plants, animals, fungi and microorganisms and the carbon stock and processes, and the nutrient stocks than forest growing on former agricultural soils. This unexpected pattern may be caused by the legacy effects caused by high nitrogen pools left by agricultural practices (Matlack, 2009; Potter et al., 2010). Nitrogen commonly limits plant growth (Du et al., 2020; LeBauer & Treseder, 2008), hence we hypothesize that the high concentrations of plant available nitrogen

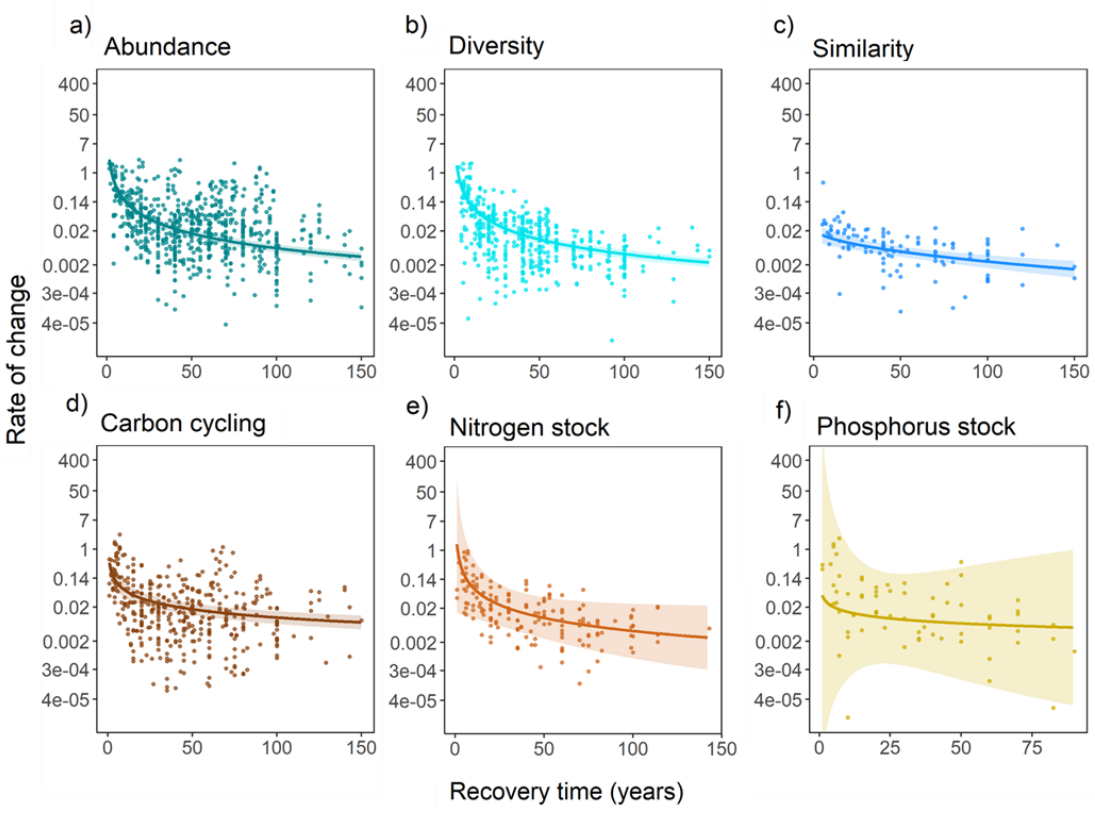

Figure 3.4. Rate of change during forest recovery across recovery metrics: a) organism abundance, b) species diversity, c) similarity, d) cycling of carbon, e) nitrogen stock and f) phosphorus stock. Rates of change are plotted on a logarithmic scale. Thick lines indicate the results of the fitted model and shadowed areas include the 95% confidence intervals of the fixed effects.

existing in post-agricultural soils accelerate the recovery of plant communities, by facilitating seedling survival and growth, and the linked carbon accumulation rates and processes caused by biomass production and decomposition (Walters & Reich, 2000).

After 150 years, community composition recovered less than the abundance of plants, animals, fungi and microorganisms. These results align with previous studies reporting that recovery of diversity metrics may require slightly over 100 years (Isbell et al., 2019; Spake et al., 2015), while the re-assemblage of species composition may require several centuries (Curran et al., 2014; Dunn, 2004; Rydgren et al., 2020). However, our predictions suggest that similarity rate of recovery was higher and may require less time to recover reference species composition than the organism abundance or species diversity. After more than a century, similarity was still far from recovery, preventing us from detecting a flattening slope in the recovery trajectory as it got closer to reference values. The slower recovery of non-woody plants populations and species composition might be explained by a difficulty for herbs to reach reference abundances and composition once decades past and the young open forest, dominated by pioneer herbs, has evolved to a close state dominated by late successional shrubs and trees (Naaf & Kolk, 2015). These results make old-growth forests an irreplaceable biodiversity resource, essential for the conservation of certain specialist species (Martin et al., 2013; Spake et al., 2015).

Our outcomes also reveal that during the first decades of recovery, times of great instability, the rate of change of all the recovery metrics is higher than in latest stages of the recovery process, when the ecosystem is closer to reference values. This might be an indicator of an increase of ecosystem stability through time and of the crucial role that the first decades of recovery play on ecosystem restoration.

Our results include a limited amount of metrics in forests recovering from agricultural or logging impacts (Table S1.1). We hypothesize that our results are conservative estimates of the actual impacts and subsequent recovery times. Metrics involving more complex ecosystem

attributes, like below ground interaction networks and linked biogeochemical functions related to the cycling of carbon and nutrients (Bardgett & Van Der Putten, 2014), will require a less likely combination of elements (e.g. phenological synchronicity, trait match) that would lead to longer recovery times (Moreno-Mateos et al., 2020). Furthermore, 36% of the selected studies included the last available data-point under recovery for at least 100 years as the reference forest, probably implying showing shorter recovery times than those happening when old-growth forests or pre-disturbance states are used as reference systems.

If forests degraded by anthropogenic impacts may need >400 years to recover some of their lost biodiversity and functionality, large-scale restoration strategies must work at those timescales. Currently, restoration strategies work with target operating at decadal scales, which would likely limit the performance of forest restoration that could translate in centennial reductions of biodiversity and ecosystem functional and services (Rodríguez-Uña et al., 2020). However, strategic planning within those strategies at the scales we are proposing will ensure an unprecedented level of commitment, which paired with aggressive capacity building and restoration science (United Nations, 2020) may allow us to truly restore forests.

Chapter 4
The long-term recovery of tree-mycorrhizal fungi interactions in European beech forests after mining[2]

Xerocomellus chrysenteron

[2] Rodríguez-Uña A, Salcedo I, Rodríguez-Echeverría, S, Moreno-Mateos, D. Centennial recovery of mycorrhizal interactions in European beech forests after mining. *Restoration Ecology.* Under revision.

Abstract

Ecological restoration strategies are emerging globally to counteract biodiversity loss and ecosystem degradation. However, restored ecosystems may not reach undisturbed biodiversity and functionality. One reason of this limited success may be a focus on short-term recovery of diversity, composition, or isolated functions. These metrics do not necessarily represent attributes less resilient whose changes operate at ecological timescales. Thus, studies of more complex metrics, like biotic interactions, at larger timescales, are essential to understand ecosystem recovery.

Using molecular identification, we estimated the recovery of the interactions between ectomycorrhizal (EcM) fungi and European beech (*Fagus sylvatica* L.) in two open-cast iron mines in use since the 14th century and abandoned over 107 and 148 years.

Species richness, species diversity and taxonomic diversity of EcM fungi recovered to undisturbed values, whereas species identity was still different. *Basidiomycota* and *Ascomycota* abundances and certain fungal functional traits (i.e. exploration and sporocarp types) also reached undisturbed values. Each location was associated to different beech-EcM fungi interactions, being *Laccaria laccata*, *Entoloma bryorum* and *Cenococcum geophylum* the indicator species for mine 1, mine 2 and outside the mines, respectively. This signal of old mining operations may be partially caused by their long-term impact on soil chemistry, as we found that the differences in soil pH and NH_4 influenced the communities of beech associated EcM fungi.

Synthesis and applications. Our results indicate that mycorrhizal interactions require >150 years to recover after the end of mining operations. These outcomes suggest that recovery metrics with more ecological information may be still capturing signals of incomplete recovery as they may better incorporate the assemblage of ecosystem complexity. Monitoring changes in recovery metrics that are less resilient to recovery and thus more accurate measurements of change, like the identity of plant-mycorrhizal fungi interactions, is critical to

evaluate the performance of restoration. Restoration efforts could then favour interactions existing in late stages of ecosystem recovery to accelerate this process. Assessing ecosystem recovery with metrics incorporating more complexity may also help to better predict time to recovery and provide deeper insights into the real magnitude of ecosystem degradation.

Resumen

Diversas estrategias de restauración de ecosistemas están emergiendo a nivel global, con el fin de contrarrestar la pérdida de biodiversidad y la degradación de los ecosistemas. Sin embargo, los ecosistemas restaurados pueden no alcanzar la biodiversidad y funcionalidad de los ecosistemas no perturbados. Una de las razones de este éxito limitado de la restauración podría ser el hecho de centrarse en la recuperación a corto plazo de la diversidad, la composición o determinadas funciones aisladas. Estas métricas no representan necesariamente los atributos menos resilientes, cuyos cambios operan en escalas de tiempo ecológicas. Por ello, aquellos estudios de medidas más complejas, como las interacciones bióticas, son esenciales para entender la recuperación de los ecosistemas.

*Mediante identificación molecular, se estimó la recuperación de las interacciones entre los hongos ectomicorrícicos (EcM) y el haya europea (*Fagus sylvatica *L.) en dos minas de hierro a cielo abierto, en uso desde el siglo XIV y abandonadas hace más de 107 y 148 años.*

La riqueza y diversidad de especies y la diversidad taxonómica de hongos EcM han recuperado los valores de la zona no perturbada, mientras que la identidad de las especies sigue siendo diferente. Las abundancias de Basidiomycota y Ascomycota y de ciertos rasgos funcionales fúngicos, es decir, el tipo de exploración y de esporocarpo, también han alcanzado los valores encontrados en la zona no perturbada. Cada ubicación se asocia a diferentes interacciones haya-hongos EcM, siendo Laccaria laccata, Entoloma bryorum y Cenococcum geophylum las especies indicadoras de la mina 1, mina 2 y el exterior de las minas, respectivamente. La señal de

las antiguas explotaciones mineras podría estar causada parcialmente por su impacto a largo plazo en la química del suelo, ya que nuestros resultados sugieren que las diferencias en el pH y el NH_4 del suelo han influido en las comunidades de hongos EcM asociados al haya.

Síntesis y aplicaciones. Estos resultados indican que se requieren más de 150 años para que las interacciones micorrícicas se recuperen tras el cese de la actividad minera. Esto sugiere que las métricas de recuperación con más información ecológica podrían estar capturando todavía señales de una recuperación incompleta, ya que puede que incorporen mejor el ensamblaje de la complejidad del ecosistema. El seguimiento de los cambios en las métricas de recuperación que son menos resilientes a la recuperación y, por lo tanto, mediciones más precisas del cambio, como la identidad de las interacciones entre plantas y hongos micorrícicos, es fundamental para evaluar el rendimiento de la restauración del ecosistema. Los esfuerzos de restauración deberían orientarse a favorecer las interacciones propias de estados tardíos de recuperación para así acelerar este proceso. La evaluación de la recuperación de los ecosistemas con métricas que incorporan una mayor complejidad podría también ayudar a predecir más adecuadamente el tiempo requerido para la recuperación y aportar conocimientos acerca de la magnitud real de la degradación de los ecosistemas.

4.1. Introduction

The accelerating pace of ecosystems' degradation compromises their ability to maintain the current levels of biodiversity, functions and ecosystem services (Cardinale et al., 2012; Newbold et al., 2015). In the last decades, ecological restoration has become a key tool to counteract this trend (Strassburg et al., 2020), as has been acknowledged with the declaration of 2021–2030 as the United Nations Decade of Ecosystem Restoration (UNGA, 2019). However, restored ecosystems may be less functional and diverse than those preserved (Moreno-Mateos et al., 2017). One of the reasons that might help explain this reduced success is that traditional restoration approaches are based on the recovery of simple attributes (e.g. endangered species population or species diversity), or single functions (e.g. reduction of soil erosion) (Montoya et al., 2012; Moreno-Mateos et al., 2017). These simplified metrics are only measured for a few years after disturbance ends, ignoring that ecosystem recovery may take centuries (Cole et al., 2014; Curran et al., 2014).

In the last years, an increasing number of studies are emphasizing the need to focus on restoring biotic interactions, as they play a key role on the structural and functional recovery of ecosystems (Kaiser-Bunbury et al., 2017; Montoya et al., 2012; Moreno-Mateos et al., 2020). One of the most relevant interactions in forest ecosystems, except tropical ones, is the mutualistic interaction between woody plants and ectomycorrhizal (EcM) fungi. It is currently estimated that *c.* 6000 plant species are involved in the association with *c.* 20,000 – 25,000 EcM fungi species of *Basidiomycota* and *Ascomycota* (Heijden et al., 2015). These fungi form a mantle external to the root of the ecologically and economically important forest trees, enhancing plant water and nutrient access and stress tolerance (Tedersoo et al., 2010). In temperate and boreal forests, low numbers of woody species are dominated by rich and diverse EcM fungal communities (Buée et al., 2005). This high diversity also exists in terms of their functional traits, like the differentiation of mycelium exploration types (Agerer, 2001) or

the type of sporocarp. It has been widely recognized that the diversity of EcM fungal communities, as well as their structure and dynamics, are strongly influenced by climatic and abiotic factors, like soil pH and nutrient levels (Hawkins et al., 2015; Lilleskov et al., 2011).

Previous studies have shown that anthropic activities such as mining or clearcutting also affect forest EcM fungal communities, by declining their richness and diversity (Gebhardt et al., 2007; Sterkenburg et al., 2019), and changing their species composition (Glen et al., 2008; J. K. M. Walker et al., 2012) for many years after the impact. However, like most studies on recovery from anthropogenic disturbance, they cover less than two decades. We still need a deeper understanding on the recovery process in the long-term, which will help us estimate the real time required to reach similar level structural and functional conditions to those existing before degradation started.

We studied the recovery of forest interactions over the long term (>100 years) after mining impacts. We compared the EcM fungal interactions with European beech (*Fagus sylvatica* L.) in two open cast iron mines in use since the 14th century and abandoned for >100 years, with the preserved surrounding forests. We sampled EcM fungal tips present in tree roots and characterized beech-EcM fungi interactions by using metrics with distinct levels of ecological information (i.e. species richness, species diversity, taxonomic diversity and species composition). We used different dating tools to estimate the moment when mining activities ceased in each mine. Our objectives were: (i) to evaluate the differences in recovery completeness among the several metrics quantifying beech-EcM fungi interactions; (ii) to assess the effect of soil conditions derived from mining operations on the recovery of beech-EcM fungi interactions; (iii) and to identify if certain phyla (i.e. *Basidiomycota* and *Ascomycota*) or fungal functional traits (i.e. exploration type and sporocarp type) are more prevalent in disturbed or preserved forests.

4.2. Methods

Study area

The study area is located in the European beech forest of Artikutza (3.638 ha), northern Spain (43°10'56.6"N 1°47'41.2"W; Figure 4.1). This area has a temperate oceanic climate, with a mean annual temperature of 12°C (19 and 6°C in the hottest and coldest months respectively) and an annual rainfall of 2500 mm (Peralta et al., 2018). The soils are mainly cambisols created from metamorphic materials, with base-metal veins, mainly iron (Galán et al., 2014). These iron deposits were exploited from, at least, the 14th century until 1919, when Artikutza was acquired by the San Sebastián City Council. Since then, no human uses (except recreational activities) were allowed, favouring forest recovery. The dominant vegetation is the Atlantic acidophilic beech forest (*Saxifrago hirsutae-Fagetum sylvaticae*; Peralta et al., 2018).

Dating mining abandonment

To estimate the moment of mining cessation we used four different tools: *i)* we searched archives with historical records of local ironworks; *ii)* we dated one sample of superficial sediments per mine, collected in 2017, using Optically Stimulated Luminescence (OSL) to estimate when they last exposed to sun radiation (Preusser et al., 2008); *iii)* we dated a charcoal sample found at the original surface after abandonment in mine 1 using accelerator mass spectrometry on the ^{14}C fraction of the sample; and finally, *iv)* we determined the age of each sampling tree using dendrochronological techniques. In spring of 2017, one core was extracted from each sampling tree and they were sanded and cross-dated, by counting each annual ring and matching ring-width patterns with Coorecorder/CDendro software (Larsson, 2018).

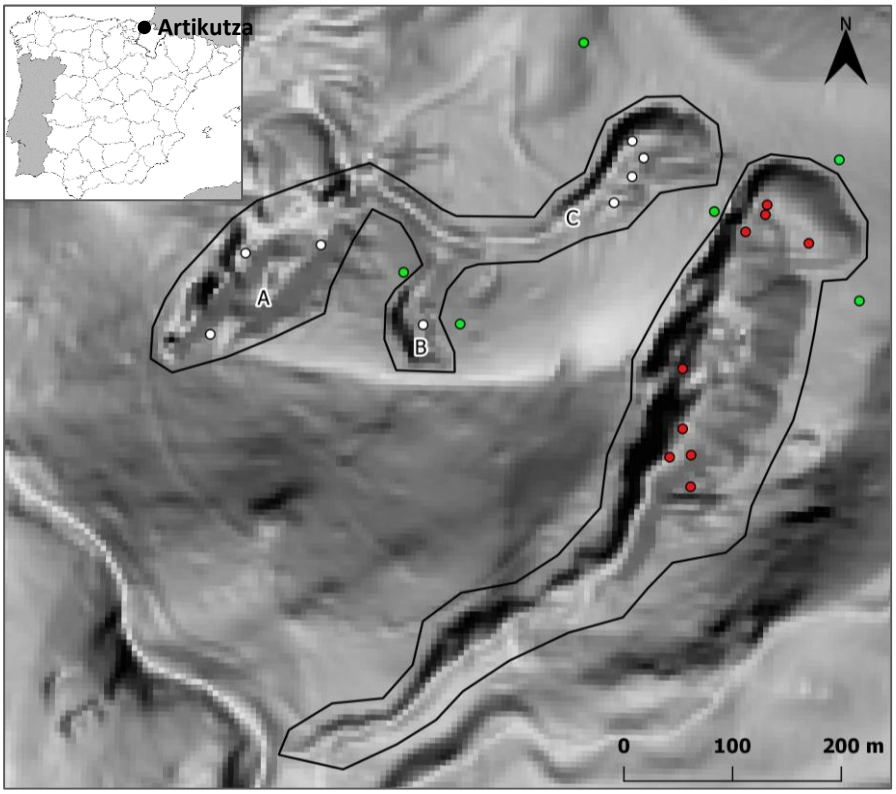

Figure 4.1. Location of the study area (Artikutza, northern Spain) and the 23 sampling trees. Red dots indicate trees inside mine 1; white dots, trees inside cuts A, B and C of mine 2; and green dots, trees outside both mines.

Sampling and processing

To study the recovery of the EcM fungal interactions with European beech trees, we selected two abandoned open cast iron mines. Mines are located in opposite sides of the same hill, whose altitude range from 250 to 750 m a. s. l. Mine 1, oriented to the SE, covers 7.7 ha. Mine 2, oriented to the NW, is divided in three cuts and covers 4.3 ha. We found three main limitations to select trees inside the mines. First, the varying and steep slopes (up to 64%) could affect EcM associations with trees (Zhang et al., 2013). To prevent this, we only selected trees growing in slopes below 40%. Second, to cover the whole time period since the end of mining, we selected the largest trees without decaying evidence in

each mine (diameter at breast height >40 cm). We finally selected nine trees inside mine 1 and eight inside mine 2 (Figure 4.1). We also sampled six trees outside the mines, adding to previous criteria that they had to be located between 10 and 100 m away from the boundaries of the mines, avoiding trees near to old mineral extraction roads.

We collected fine roots from each tree in spring and autumn of 2017. In each campaign, we collected 500 ml of roots from four plots 25x25 cm^2 and 10 cm deep. The plots were 1.5 m away from the tree and at 90° from each other. We rotated the points 45° to the right in autumn to avoid collecting roots from the same plot. To preserve the roots, we kept them with their own soil in plastic bags at 4° C, until they were processed within the following 48 hours. From each sample, we randomly selected five 5-cm root segments. Using stereo-microscopes, we identified and quantified the EcM morphotypes following Agerer (1987) and Agerer (2001). In order to maximize the resolution of EcM identification, at least one tip of each morphotype distinguished in each segment was preserved in hexa-decyl-trimethyl-ammonium bromide (CTAB) for later DNA extraction and sequencing. We also collected 100 g of soil at each of the four plots from each tree to estimate the soil carbon and nutrient concentration. Soil samples were dried at 40° C for 48 hours and homogenized. Samples were then analyzed using spectrophotometry to estimate the concentration of ammonium (NH_4), nitrate (NO_3) and Olsen phosphorus (P) (Olsen, 1954). The percentage of organic carbon (C), using the Walkley and Black (1934) method, and soil pH were also determined.

EcM identification and sequence bioinformatics

We sequenced the DNA of 691 EcM root tips following a modification of Murray & Thompson (1980) protocol. ITS1F-ITS4 primers were used for PCR amplification of the ITS rDNA region (White et al., 1990). A semi-nested PCR (with ITS1F-ITS2 or ITS3-ITS4 primers) was needed for amplifying partial fragments in 14% of the sequences where the initial reaction failed. We also repeated the PCR for 8% of the sequences with basidiomycete-specific primers ITS1F-ITS4B (Bellemain et al., 2010),

when the first protocol did not render good quality reads. When this reaction failed, a semi-nested PCR was carried out with ITS3-ITS4B primers. Amplification program consisted in a hot start at 95°C for 5 min, followed by 35 cycles of 45, 30 and 45 sec at 94°C, 54°C and 72°C, respectively; and a final elongation phase at 72°C for 10 min. Results were checked in an agarose gel at 1%, and positive reactions were purified and sequenced with ITS1F-ITS4. All sequences and chromatograms were checked to detect and correct reading mistakes.

We used Geneious Prime 2019.0.4 (https://www.geneious.com) to analyze 598 positive DNA sequencing results. Low quality ends were trimmed, prior to filtering by sequence quality. The 464 resultant sequences were matched against GenBank using BLAST (https://www.ncbi.nlm.nih.gov/BLAST), with a similarity threshold of 97% (Anslan et al., 2016). We rejected 16 sequences that matched non-EcM fungi and 18 sequences that matched order *Helotiales,* considered ericoid mycorrhizal fungi (Tedersoo et al., 2009). When one sequence was identified with two or more *species affinis* (i.e. closely related, but not identical species), we selected the species already identified in nearby beech forests. The insufficient resolution of the ITS region to identify the species inside certain genera may be one of the reasons why 57 sequences (12%) were not identified at the species level. To reduce this proportion, they were aligned with the other sequences of their genus. As genus *Tomentella* had the highest proportion (20 from a total of 27) of OTUs not affiliated to a taxonomically identified species, we ran a maximum likelihood phylogeny with one representative sequence of each potential species and their closest matching sequences at GenBank (see phylogenetic placement of sequences in Figure S4.1), using MEGA X (Kumar et al., 2018). Finally, we discarded the remaining 21 sequences not identified at species level, resulting in a final dataset of 409 DNA sequences, which were deposited in the NCBI GenBank (accession numbers MW282331 - MW282739; Table S4.2).

Statistical analysis

We examined the differences in species richness, species diversity (Shannon-Wiener index) and taxonomic diversity between mine 1, mine 2 and outside of the mines. The three metrics were calculated using the package *vegan* (Oksanen et al., 2019) in R 3.6.3 (R Core Team, 2020). Taxonomic diversity was measured as the average taxonomic distinctness (Δ^+) between pairs of species of each sampling tree (Clarke & Warwick, 1998) (see dendrogram of taxonomic distances in Figure S4.2). We fitted a linear mixed model for species diversity and taxonomic diversity, with location (mine 1, mine 2 and outside) and sampling season (spring vs. autumn) as fixed effects and sampling trees as random effect, using the function *lmer* of R package *lme4* (Bates et al., 2015). For species richness, we used the function *glmer* of R package *lme4* to fit a generalized linear mixed model with the same fixed and random effects but choosing a Poisson error distribution. We compared the models based on the corrected Akaike Information Criterion (AICc; (Burnham & Anderson, 2002)), selecting the most parsimonious one with ΔAICc ≤2 in comparison to the minimum AICc among all models.

We tested the influence of location in each soil variable (C, NH_4, NO_3, P and pH) using analysis of variance (ANOVA) with post-hoc Tukey pairwise tests. NH_4, P and pH data were transformed logarithmically to satisfy the criteria of normal distribution and homogeneity of variances. We determined whether these soil variables were correlated to each other by estimating the pairwise Pearson's regression coefficient. We also fitted linear regression models including all soil variables to test their effect on species richness, species diversity and taxonomic diversity.

We assessed the effect of location and sampling season on beech-EcM fungi interactions, first converted into a Bray-Curtis dissimilarity matrix, with a permutational multivariate analysis of variance (PERMANOVA). We used the *adonis* function of the R package *vegan*, with 9999 permutations. To visualize the similarity of beech-EcM fungi interactions between the trees in mine 1, mine 2 and outside, we

generated a non-metric multidimensional scaling plot (NMDS) using the Bray Curtis similarity index in the *metaMDS* function of the R package *vegan*. To further assess the influence of soil variables on beech-EcM fungi interactions, we run another PERMANOVA analysis replacing the effect of location and sampling season by the effect of all soil variables (C, NH_4, NO_3, P and pH). A canonical correspondence analysis (CCA) was conducted to visualize the ordination of beech-EcM fungi interactions of mine 1, mine 2 and outside in relation to the soil variables.

We also identified those species (or combinations of them) associated with mine 1, mine 2 or outside using the indicator species analysis (Dufrêne & Legendre, 1997) from the *multipatt* function in the R package *indicspecies* (De Cáceres & Legendre, 2009), with 9999 permutations. This analysis assesses the relationship between species abundance and groups of sites, and the statistical significance of such associations. We further assessed beech-EcM fungi interactions responses to mining impact. We compared mean relative abundances of each phylum (*Ascomycota* or *Basidiomycota*) and two functional traits: exploration type (contact-short, medium or long) and sporocarp type (corticioid, gasteroid, pezizoid, stipitate or without sporocarp) between mine 1, mine 2 and outside using an ANOVA with post-hoc Tukey pairwise tests. Assignments of exploration type were based on Agerer (2001, 2006), Ostonen et al. (2017) and Defrenne et al. (2019).

4.3. Results

Recovery time estimation

OSL results revealed that mine 1 and cut C of mine 2 (Figure 4.1) were abandoned 138 ± 25 and 125 ± 15 years, respectively, before the samples were collected. The historical records showed that cut A and B of mine 2 were abandoned in 1910 (Moreno Mateos et al., 2019). The age of the charcoal sample from mine 1 was estimated in 128 ± 38 years. The dendrochronological study of the sampling trees showed ages of 67 to 148 and 78 to 144 (min. – max.) inside mines 1 and 2, respectively.

These results suggest that mine 1 and cut C of mine 2 were abandoned between 125 and 148 years before sampling, and cut A and B of mine 2 were abandoned 107 years before sampling.

Recovery of interactions and functional traits

The obtained 409 sequences corresponded to 98 EcM fungal OTUs (hereafter referred to as 'species') within 39 genera across the 23 sampling trees inside and outside the mines. The most abundant genera were *Tomentella*, *Cenococcum*, *Entoloma* (only present in the mines), *Lactarius*, *Inocybe*, *Russula* and *Sebacina* (Figure S5.1), which have been identified in nearby beech forests (Goicoechea et al., 2009; Sarrionandia et al., 2009). The amount of unique species detected out of the total amount of species in each location was 21 out of 56 in mine 1, 25 out of 54 in mine 2 and 29 out of 54 outside. Mine 1 and mine 2 share eight species, mine 1 and outside share 10 species, mine 2 and outside share 3 species and the three locations share four species (Table S5.1). *Cenococcum geophilum* was the most common species in all locations, with a relative abundance of 7.88% ± 5.04%, 7.85% ± 3.86% and 24.18% ± 7.47% (mean ± s.e.) in mine 1, mine 2 and outside, respectively. The indicator species analysis considered *C. geophilum* (p = 0.01; Table S5.2) as the best indicator species of beech-EcM fungi interactions present outside the mines. Other abundant species only present outside the mines were *Sistotrema sp.1*, *Lactarius blennius* and *Russula fellea*. The most abundant species appearing inside both mines but not outside were *Entoloma bryorum*, *Laccaria laccata, Inocybe maculata* and *Cyanoboletus pulvurulentus*. In mines 1 and 2, the analysis selected *L. laccata* (p = 0.02) and *E. bryorum* (p <0.001), as indicator species of each mine, respectively (Figure 4.2; Table S5.2).

The species richness per tree was 5.50 ± 1.76, 6.13 ± 2.53 and 6.18 ± 2.61 (mean ± s.e.) in mine 1, mine 2 and outside, respectively. The species diversity per tree was 1.33 ± 0.34, 1.43 ± 0.27 and 1.41 ± 0.41 (mean ± s.e.) in mine 1, mine 2 and outside, respectively. The taxonomic diversity per tree was 60.26 ± 10.18, 64.48 ± 8.67 and 63.95 ± 8.76 (mean ± s.e.) in mine 1, mine 2 and outside, respectively. We found no

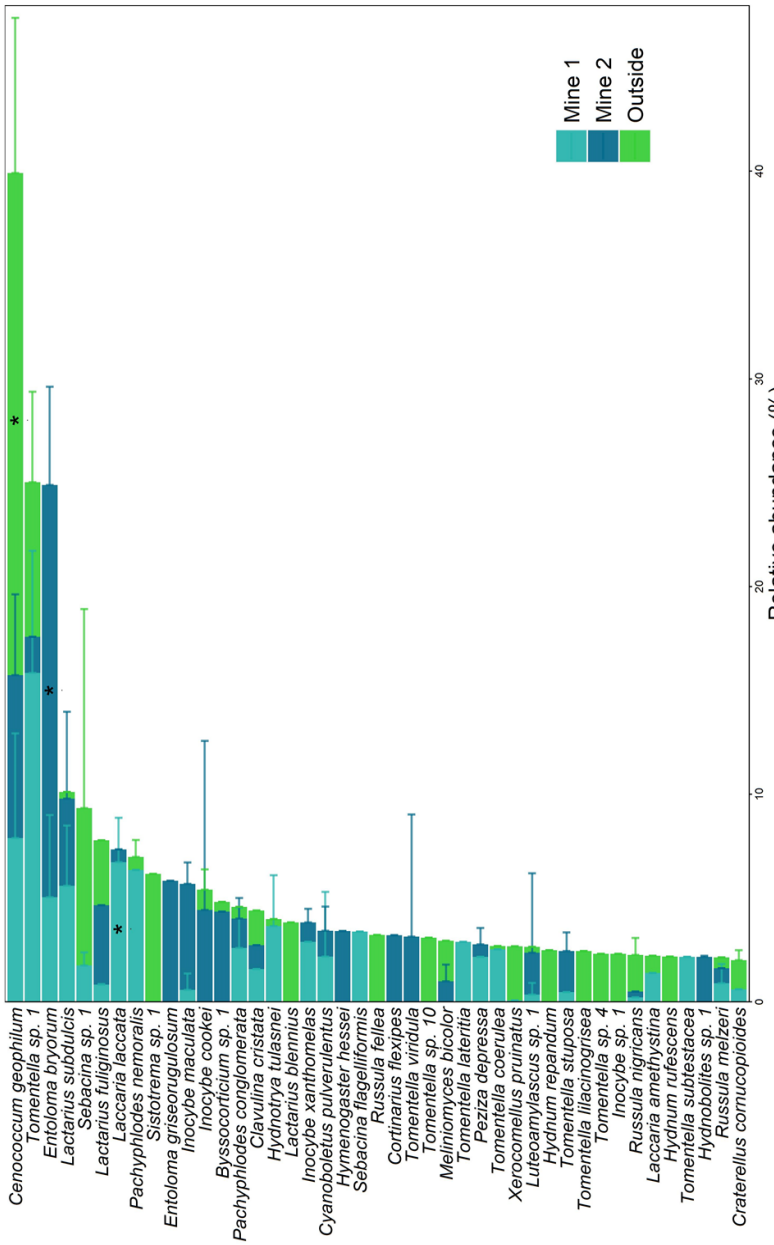

Figure 4.2. Relative abundance of ectomycorrhizal fungal species found in European beech roots with and outside both mines. It only shows species with >2% total relative abundance. Bars indicate means and standard error. Asterisks indicate the best indicator species of mine 1, mine 2 and outside as determined by the indicator species analysis.

differences in species richness, species diversity and taxonomic diversity between the three sampled locations or the sampling season, i.e. the model that excluded the effect of location and sampling season had the minimum AICc (Table S5.3).

The PERMANOVA model revealed a significant effect of location on EcM fungal communities, but not of sampling season (Table 4.1). NMDS visualization of EcM fungal communities was consistent with the effect of location (Figure 4.3). When EcM fungal species were grouped by sporocarp type, we found a higher mean relative abundance of the "stipitate" group in mine 2 with respect to mine 1 and outside (ANOVA: $F_{2,20} = 11.40$, $p = 0.05$; followed by post-hoc Tukey pair-wise tests; Figure S5.2a). We found no differences in the mean relative abundances of EcM fungal species among locations when grouped according to their phylum and mycelium exploration type (Figure S5.2b; Figure S5.2c).

Table 4.1. Results of the PERMANOVA analysis to test the effect of location (mine 1, mine 2 and outside the mines) and sampling season (spring and autumn), and soil variables on ectomycorrhizal fungal communities found in European beech roots.

Location & sampling season					
Source	**Df**	**Sum Sq**	**Pseudo-*F***	**R^2**	***p*(perm)**
Location	1	1.17	2.75	0.06	**<0.001**
Sampling season	1	0.47	1.09	0.02	0.31
Residuals	43	18.38		0.92	
Total	45	20.02		1.00	
Soil variables					
Source	**Df**	**Sum Sq**	**Pseudo-*F***	**R^2**	***p*(perm)**
pH	1	0.83	2.01	0.09	**0.002**
NH_4	1	0.53	1.27	0.05	0.13
NO_3	1	0.34	0.83	0.03	0.81
C	1	0.43	1.04	0.04	0.42
P	1	0.45	1.08	0.05	0.34
Residuals	17	7.03		0.73	
Total	22	9.60		1.00	

Df: degrees of freedom; Sum Sq: sum of squares; Pseudo-*F*: *F* value by permutation, *p*(perm): *p*-values based on 9999 permutations (statistically significant *p*-values are shown in bold).

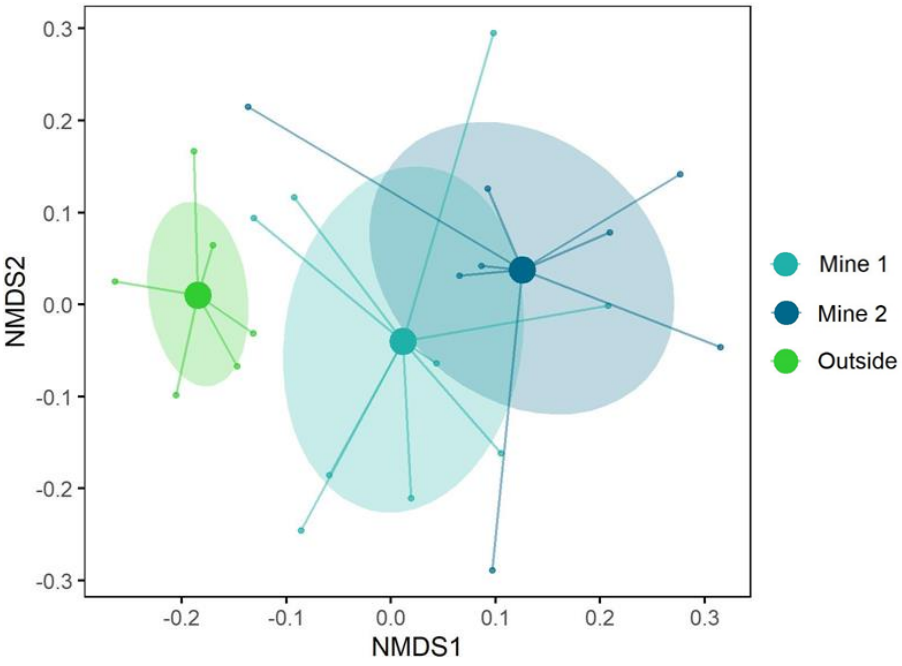

Figure 4.3. Non-metric multidimensional scaling (NMDS) plot of the ectomycorrhizal fungal communities found in European beech roots inside and outside mines (stress value = 0.19). Points on the ordination space represent sampling trees based on Bray-Curtis dissimilarity indices. Centroids and standard deviation ellipses of the three locations are overlaid.

Changes in soil biogeochemistry

Soils from the three sampled locations had different NH_4 (ANOVA: $F_{2,20}$ = 11.40, p <0.001) and P (ANOVA: $F_{2,20}$ = 3.78, p = 0.04) concentrations and pH ($F_{2,20}$ = 11.39, p <0.001) values (Figure 4.4). However, C and NO_3 concentrations were not different among sites (Figure 4.4; Table S6.1). Post-hoc Tukey pairwise tests detected a higher pH and lower NH_4 in mine 2 with respect to mine 1 (pH: p = 0.005, NH_4: p <0.001) and outside (pH: p <0.001, NH_4: p <0.001). In contrast, P was higher in mine 1 compared to mine 2 (p = 0.02) and to outside (p = 0.03). NH_4 and pH were negatively correlated (ρ = -0.46, p = 0.03; Figure S6.1). We found no effects of soil variables on species richness, species diversity or taxonomic diversity (Table S6.2).

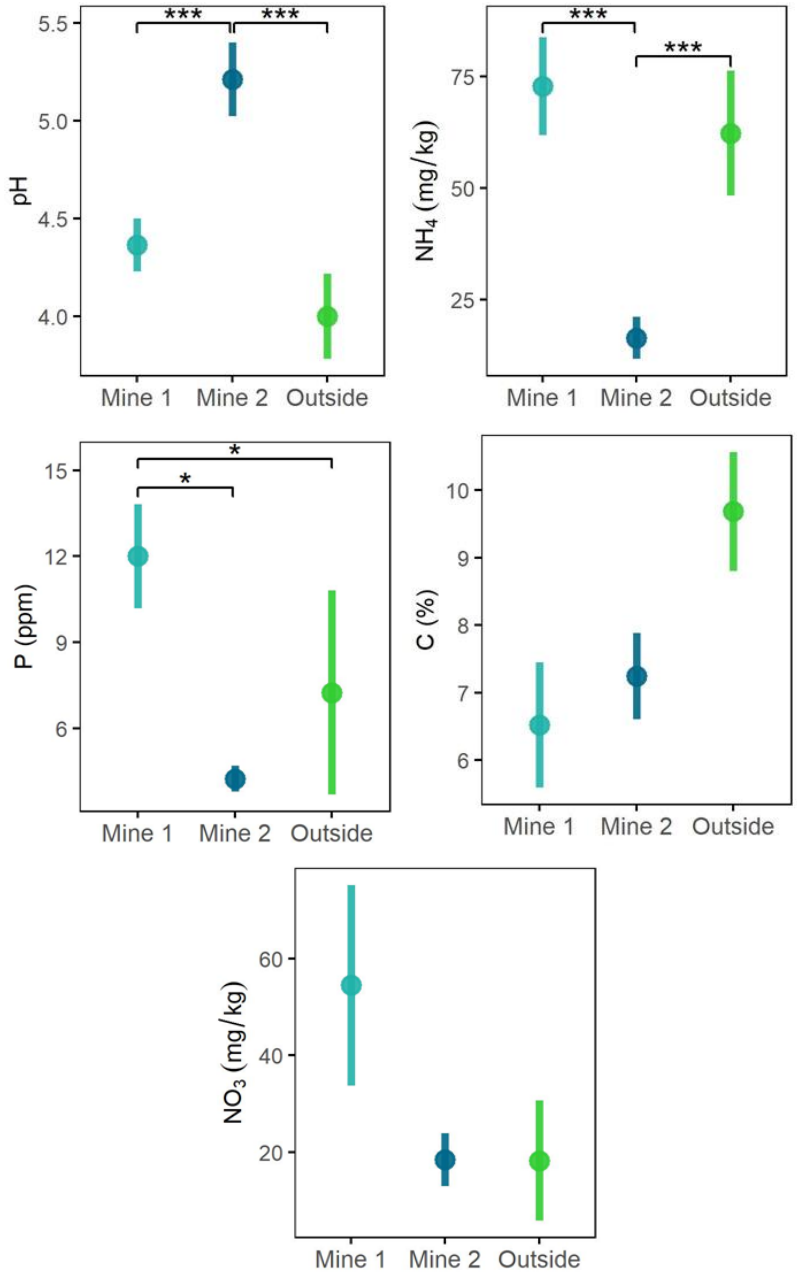

Figure 4.4. Mean value (± standard error) of the soil variables measured with and outside mines. Asterisks indicate significant differences (*: *p*-value <0.05, ***: *p*-value <0.001) obtained from Tukey post-hoc pairwise comparisons of the ANOVA results from Table S6.1.

The PERMANOVA model indicated that pH but not C, NH_4, NO_3 and P influenced EcM fungal communities (Table 4.1). CCA visualization shows a separation of EcM fungal communities of mine 2 from mine 1 and outside, mainly explained by the effect of pH, followed by an effect of NH_4 (Figure 4.5; Table S6.3).

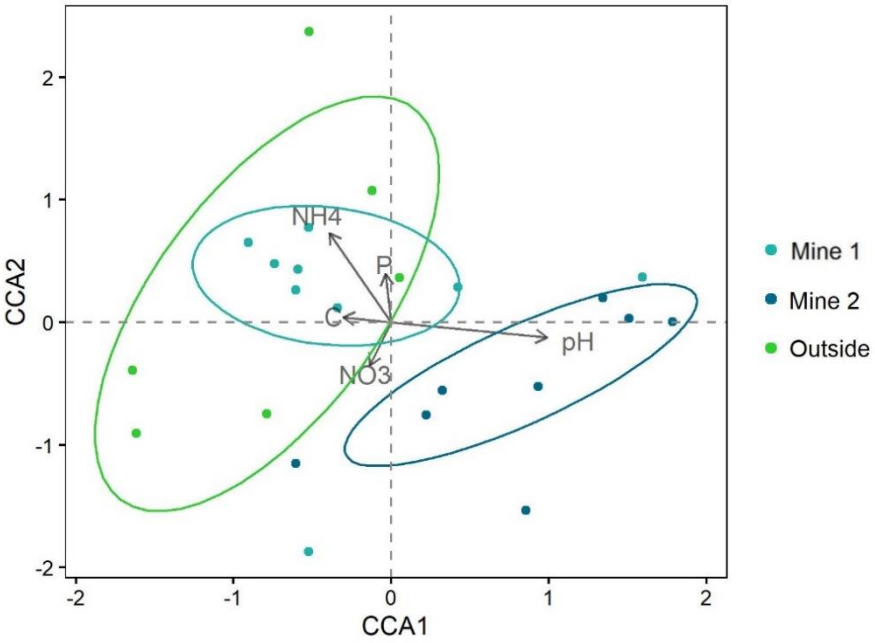

Figure 4.5. Canonical correspondence analysis (CCA) plot of the ectomycorrhizal fungal communities found in European beech roots inside and outside mines. Points in the ordination space represent sampling trees based on the effect of the soil variables measured (C, NH_4, NO_3, P and pH). Standard deviation ellipses of the three locations are also overlaid.

4.4. Discussion

Our results reveal that, after >148 and >107 years of open cast mining abandonment in mine 1 and mine 2, respectively, the species richness, species diversity and taxonomic diversity of EcM fungi associated with beech trees have recovered to values present in the preserved surrounding forest. However, the identity of beech-EcM fungi interactions was different in former mines. Previous studies found

similar results for species richness of EcM fungi associated with *Quercus rubra* which recovered up to 63% after 43 years since mining cessation (Gebhardt et al., 2007). EcM fungal species richness on jarrah forest recovered in 16-year-old rehabilitated mine sites but species composition did not (Glen et al., 2008). A meta-analysis from temperate, Mediterranean and boreal planted or secondary forests estimated that EcM fungal richness required on average 90 years (between 45 years to unrecoverable at 95% prediction limits), to reach old-growth forests' values (Spake et al., 2015). More broadly, another meta-analysis on forest reported that species richness in restored forests may converge to reference levels within a century, while the recovery of species composition may take up to an order of magnitude longer (hundreds to thousands of years) (Curran et al., 2014).

Although both mines share EcM fungal species with the preserved surroundings, some species that are abundant either inside or outside the mines are not present in the counterpart location. These dissimilarities may be driven by different abiotic conditions and distinct forest-recovery stages. For example, *L. laccata*, known for its ability to assimilate NO_3 (Hobbie et al., 2008), was the indicator species in mine 1, where we found the highest concentration of NO_3. Beech-EcM fungi interactions outside the mines are characterized by *i)* the dominance of *C. geophilum*, a generalist in temperate forests (Douhan & Rizzo, 2005); *ii)* the presence of acidophilic species, like *Lactarius blennius* (Sarrionandia et al., 2009); and *iii)* an increasing abundance of species of the genus *Russula*, commonly found in mature forests (Twieg et al., 2007). These results might indicate that mycorrhizal interactions inside the former mines have not recovered yet to our reference stage. Other studies based on temperate and boreal forests also demonstrated an evolution towards old-growth EcM fungal communities during secondary forest succession (Spake et al., 2016; Twieg et al., 2007).

The higher similarity of EcM fungal communities between mines compared to the surrounding preserved forest, together with the effect of location (i.e. mine 1, mine 2 or outside the mines) on those interactions, suggests that previous mining impacts explain part of the

differences in the interaction identity. To test the influence of previous mining impacts, it would have been preferable to include replicate sites from other mined forests. Unfortunately, the uniqueness of the study area makes it ideal for studying long-term recovery but hampers the possibility to find other sites with similar environmental conditions and resource exploitation approaches. These are sites affected by the same impact and for the same period of time, recovering for >100 years. Nevertheless, the dissimilarity between EcM fungal communities from disturbed and preserved trees was higher for mine 2 than for mine 1. We propose two ways to explain this. First, the difference in mining ending dates between mines: activities ended later in cut A and B of mine 2 (in 1910) compared to cut C (in 1892 ± 15 years) and mine 1 (in 1879 ± 25 years). Second, the difference in soil chemistry: values of pH and NH_4 were different in mine 2 compared to mine 1 and outside the mines. Values of pH and NH_4 were the strongest determinants of EcM fungal communities in our study area. This explanation is further supported by the strong effect of pH on EcM fungal communities found in the PERMANOVA analysis. These findings parallel other studies documenting a major influence of pH and NH_4 on EcM fungal community composition (Hawkins et al., 2015; Lilleskov et al., 2011), but with no effect on species richness (Toljander et al., 2006).

Our results also show that none of the phyla or functional traits, except for the "stipitate group", are associated to inside or outside the mines, i.e. disturbed and not disturbed areas. In contrast, other studies documented differences in the abundance of hypogeous and epigeous fungi among forest-recovery stages (Durall et al., 2006; Smith et al., 2002). However, these studies were based on observed sporocarps instead of EcM sampled *in planta*. Overall, our findings suggest that the effect of mining on the proportion of *Ascomycota* and *Basidiomycota* fungi, the strategies of mycelium exploration and the type of sporocarp recovered after >107 and >148 years of mine abandonment.

Our results suggest that while ecosystems may seem recovered over the long term based on metrics with low levels of ecological information, like those measuring the number and abundance of species, actual

recovery may not happen. We have found that diversity recovered, while the identity of plant-mycorrhizal fungi interactions did not recover after >148 years. Metrics with more ecological information may better incorporate the assemblage of ecosystem complexity (Moreno-Mateos et al., 2020; Rydgren et al., 2020). Monitoring changes in these more complex metrics after restoration will likely provide a more accurate estimate of its performance. Restoration efforts could then favor beech-EcM fungi interactions existing in late stages of forest recovery to accelerate this process. To improve this knowledge, more studies estimating how much nitrogen is absorbed by the plant thanks to each EcM fungal species (Pena et al., 2013), would enable to identify highly functional species or clusters of species to be used in forest restoration (Hawkins et al., 2015). In addition, assessing ecosystem recovery with metrics that incorporate more ecosystem complexity may also help to better predict the time required to full recovery and provide deeper knowledge about the real magnitude of ecosystem degradation.

Chapter 5
The long-term recovery of Collembola-soil fungi interaction networks after mining

Abstract

Despite the dramatically growing policy support and practice of ecological restoration, its performance and outcomes are still unclear. This is particularly relevant at ecological timescales (centuries or more) and with ecosystem components involving high ecological complexity (many organisms and functions involved) that may also be less resilient, like those existing belowground.

We analysed the recovery of Collembola-fungi interactions in two open-cast iron mines in use since the 14th century and abandoned for >107 and >148 years. Collembola and soil fungi play key roles in soil ecosystem functioning (e.g. decomposition, nutrient absorption, pathogenesis, plant communication) and are vulnerable to soil degradation. We sampled Collembola communities around 23 European beech (*Fagus sylvatica* L.) trees inside and outside the mines to construct a qualitative trophic interaction network for each tree. We identified the Collembola-associated fungi in their gut contents using DNA meta-barcoding.

Our results show that while Collembola species richness and diversity have recovered inside the mines, their species composition remains different from the preserved surroundings. This may be partly due mining legacies resulting in elevated soil pH. Collembola-associated fungal communities inside and outside the mines also differ in their functional groups, mutualistic and saprotrophic interactions were more frequent than parasitic interactions outside the mines. Networks from undisturbed forests present unique species and compartmentalized and weakly connected architectures that make them more robust to extinctions than networks within the former mines.

Synthesis and applications. Our results indicate that Collembola-fungi interaction networks require >150 years to recover after the end of mining operations. We suggest that actions to accelerate the recovery process should include restoring species with crucial roles in re-establish network modularity, which will foster the rebuilding of below-

ground trophic networks. Evaluating the reestablishment of the architecture of interaction networks, and associated community structure and biogeochemical functions, is essential to understand the performance of restoration and better predict the time to recovery.

Resumen

A pesar de que el apoyo político y la práctica de la restauración ecológica han aumentado enormemente, su rendimiento y resultados siguen sin estar claros. Esto es particularmente relevante para escalas de tiempo ecológicas (siglos o más) y para componentes del ecosistema que incluyen una alta complejidad ecológica (involucran a muchos organismos y funciones) que además pueden ser menos resilientes, como los presentes bajo tierra.

*En este capítulo analizamos la recuperación de las interacciones colémbolos-hongos en dos minas de hierro a cielo abierto, en uso desde el siglo XIV y abandonadas durante más de 107 y 148 años. Los colémbolos y los hongos edáficos juegan un papel clave en el funcionamiento de los ecosistemas edáficos (p. ej. en la descomposición, la absorción de nutrientes, la patogénesis o la comunicación entre plantas) y además son vulnerables a la degradación del suelo. Se muestrearon las comunidades de colémbolos en torno a veintitrés hayas europeas (*Fagus sylvatica *L.), ubicadas dentro y fuera de las minas, para construir una red trófica cualitativa por cada árbol. Los hongos presentes en el contenido gástrico de los colémbolos fueron identificados mediante DNA metabarcoding.*

Nuestros resultados muestran una recuperación de las riqueza y diversidad de especies de colémbolos en las minas, mientras que la composición de especies se mantiene diferente a la de los alrededores conservados. Esto puede deberse en parte al legado de la minería, que ha dado lugar a un elevado pH del suelo. Las comunidades de hongos asociados a los colémbolos también difieren en sus grupos funcionales, ya que las interacciones mutualistas y saprobias fueron más frecuentes que las parásitas en el exterior de las minas. Las redes de bosques no

perturbados presentan especies únicas y arquitecturas compartimentadas y débilmente conectadas, favoreciendo una mayor robustez a extinciones que las redes del interior de las antiguas minas.

Síntesis y aplicaciones. Nuestros resultados indican que se requieren >150 años para la recuperación de las redes de interacción colémbolos-hongos, después del fin de las operaciones mineras. Proponemos que las acciones para acelerar los procesos de recuperación incluyan la restauración de especies cruciales para el re-establecimiento de la modularidad de la red, ya que promueven la reconstrucción de las redes tróficas hipogeas. Es esencial evaluar el re-establecimiento de la arquitectura de las redes de interacción, y de la estructura de las comunidades y de las funciones biogeoquímicas asociadas, para entender el rendimiento de la restauración y mejorar la predicción del tiempo necesario para la recuperación.

5.1. Introduction

In the last decades, ecological restoration has become a global priority to mitigate the accelerated ecosystem degradation and biodiversity loss (Gann et al., 2019; Strassburg et al., 2020). This has been recently exemplified with the declaration of the United Nations Decade of (2021–2030) of Ecosystem Restoration (UNGA, 2019) and other ambitious restoration commitments, like the New York Declaration on Forests or the European Union Biodiversity Strategy for 2030 (European Commission, 2020; NYDF Assessment Partners, 2019). Nevertheless, several assessments of the restoration performance worldwide have concluded that restored ecosystems may not get pre-disturbance levels of their structure, functions and services when traditional restoration guidelines are followed (Curran et al., 2014; Jones et al., 2018; Moreno-Mateos et al., 2017). These traditional approaches are based on the recovery of simple attributes (e.g. species richness or diversity), or single functions (e.g. soil carbon content) that do not encapsulate much ecological information. An increasing number of studies have proposed the need to move from these simple metrics towards focusing on restoring biotic interactions, which play a key role on the structural and functional recovery of ecosystems (Kaiser-Bunbury et al., 2017; Montoya et al., 2012; Moreno-Mateos et al., 2020).

During the recovery process, the gain in species may be decoupled from the gain in interactions, because interaction realization requires species to have a high probability to encounter, compatible functional traits and synchronized phenology (Morales-Castilla et al., 2015). This is particularly the case for below-ground interactions, where the recovery of interaction networks and their structure may be a more important determinant for the recovery of ecosystem functionality than the recovery of biodiversity (Montoya et al., 2015; Morriën et al., 2017). Studying the recovery of soil biota interactions, like the interaction between soil microarthropods and fungi, is crucial as they are important determinants of the recovery of ecosystem functioning (Wagg et al., 2019). Fungi play a crucial role in organic material decomposition,

mycorrhizal symbiosis with plant roots and pathogenic mechanisms (Tedersoo, Anslan, et al., 2020). Collembolas are essential components of soil mesofauna because of their role in forming soil microstructure and in litter decomposition processes (Chamberlain et al., 2006; Rusek, 1998). Collembolas may shape fungal communities and fungal functional groups (e.g. saprotrophs, parasites or mycorrhizal fungi) by restraining certain taxa due to preferential feeding and favouring other taxa via propagule dispersion and competition reduction (Anslan et al., 2016). Collembola and soil fungi are sensitive to soil degradation, making them useful indicators of the recovery of the soil-litter system (Sterkenburg et al., 2019; Sterzyńska & Skłodowski, 2018). Several studies have reported a decline of fungal or Collembola community species richness, diversity or changes in their species composition after mining (Glen et al., 2008; Zeppelini et al., 2009), logging (Chauvat et al., 2011; Sterkenburg et al., 2019), agriculture (Dou et al., 2019; McGuire et al., 2015) and prescribed fires (Dove & Hart, 2017; Malmström, 2012).

However, little is known about the post-disturbance recovery of Collembola-fungi interactions at ecological timescales. We face this limitation by addressing the recovery of Collembola-fungi interactions in two opencast iron mines, in use since the 14th century and abandoned for more than 100 years. We compared the community structure of Collembola and their trophic interactions with soil fungi that exist inside the mines with those from the preserved surrounding forest, as well as the architecture of their interaction networks. Our objectives were (*i*) to determine whether Collembola community structure recovered after >100 years of recovery after mining and to what extent soil conditions derived from mining operations affected this recovery process; *(ii)* to find differences in Collembola trophic interactions and fungal functional guilds between disturbed and preserved forests; and *(iii)* to evaluate the recovery of the architecture of Collembola-fungi interaction networks.

5.2. Materials and methods

Study area

As described in Chapter 4, our study area was located in the European beech (*Fagus sylvatica* L.) forest of Artikutza (3.638 ha) in northern Spain (43°10'56.6"N 1°47'41.2"W). It has a temperate oceanic climate, with an annual rainfall of 2500 mm. The soils are mainly cambisols, with iron veins that were exploited since, at least, the 14th century until 1919, when Artikutza was acquired by the Donostia-San Sebastián City Council. Since then, no human uses (except recreational activities) were allowed, favouring forest recovery (for a more detail description of the study area, see *Methods* in Chapter 4).

Sample collection

We collected soil samples around the same beech trees sampled in Chapter 4, in spring and autumn of 2017 (see *Methods* in Chapter 4 for complete information about mines location and trees selection). Seventeen trees were sampled inside two abandoned opencast iron mines, nine trees inside mine 1 and eight inside mine 2, and six trees were sampled outside the mines (Figure 5.1). Mine 1 covers 7.7 ha and mine 2 includes three cuts and covers 4.3 ha. Mine 1 and cut C of mine 2 were abandoned between 125 and 148 years before sampling, and cut A and B in mine 2 were abandoned 107 years before sampling (see *Methods* and *Results* in Chapter 4 for a detailed explanation about dating mining cessation). In each season, we collected at each tree 500 ml of soil from four 25x25 cm^2, 10 cm–deep plots. The plots were 1.5 m away from the tree and at 90° from each other. We rotated the points 45° to the right in autumn to avoid collecting soil from the same plot. To preserve the samples, we kept them in plastic bags at 4° C, until they were processed within the following 48 hours. We extracted the Collembolas from the soil samples using a modified Tullgren funnel for seven days into 70% ethanol solution, with incandescent light bulbs as a heat source. Afterwards, each specimen was surface sterilized with ethanol and identified as far as possible to species level and quantified

by a taxonomist (see thesis' *Acknowledgements*), using stereo-microscopes.

Dietary data generation

To characterize Collembola-associated fungi in their gut contents, prior to DNA extraction, we removed the ethanol used as a preservative from the 162 Collembola samples obtained (Saitoh et al., 2016). DNA was extracted using 500 µl of a lysis buffer and 3.15 mg of Dithiothreitol (DTT). To facilitate cell disruption, specimens were frozen at -80 °C for 4 min, then heated at 65°C for 5 min and frozen again at -80 °C for 4 min. Samples were then incubated at 56 °C for 8 h just after adding 2.5 ml of proteinase K. Extraction controls were conducted for each batch of samples. DNA concentration extracted in each sample was quantified using a Qubit 2.0 Fluorometer with a dsDNA HS assay kit (Life Technologies, Carlsbad, CA, USA) and bead-purified using streptavidin coated baits (Beckman Coulter, Brea, CA, USA). We targeted the ITS1 region by using ITS1F12 (Schmidt et al., 2013) forward and ITS2 reverse primers (White et al., 1990), as the entire ITS rDNA region is too long (typically ranges between 450 and 700 bp) for high-throughput sequencing methods (Bellemain et al., 2010). Both primers were 5′ nucleotide tagged to yield a set of unique forward and unique reverse primers. DNA extracts from gut content contain a mixture of genomic DNA from different organisms that usually is highly degraded (Taberlet et al., 2012). Thus, samples were screened using quantitative PCR (qPCR) to assess the quality and purity of the extracted DNA and determine the optimal PCR conditions (Murray et al., 2015). PCR reactions were carried out in a 25 µl volume containing 2.5 µl 10x PCR Buffer, 2.5 µl MgCl2 (2.5 mM,) 0.2 µl dNTPs (10 mM each), 1.5 µl of BSA (20 mg ml^{-1}), 0.5 µl of AmpliTaq Gold® DNA Polymerase (5 U/µl), 3 µl of primers mix (10 µM each) and 5 µl DNA extract. Amplification program consisted in a hot start at 95 °C for 10 min, followed by 36 cycles of 30, 30 and 1 min at 95 °C, 55 °C and 72 °C, respectively; and a final elongation phase at 72 °C for 10 min. 4 µl of PCR products were visualised on 2% agarose gels. An amplification score was assigned to

each PCR reaction based on gel band strengths and these scores were used to guide the pooling of PCR products. Amplicon pools were subsequently bead-purified using streptavidin coated baits and quantified using a Qubit 2.0 Fluorometer with dsDNA HS assay kit. Amplicons pools were built into libraries using a modified version of the BEST protocol (Carøe et al., 2018) adapted for metabarcoding. The molarity of each library was quantified through qPCR using the NEBNext® Library Quant Kit Amplicon libraries. The resulting libraries were sequenced in an Illumina MiSeq high-throughput sequencer with 250PE chemistry at the National High-throughput Sequencing Centre at the Natural History Museum of Denmark.

The paired end reads in each sequencing library were merged and quality filtered using AdapterRemoval 2.1.7 and the reads within each library were sorted according to primer and tag sequences using DAMe. DNA sequences were clustered into operational taxonomic units (OTUs) based on 97% similarity threshold. Taxonomy assignment was done using the Genbank database (Benson et al., 2012). We also discard those OTUs corresponding to contaminations from human skin microbiota, airborne and waterborne fungi present in lab reactives and surfaces and widespread distributed (water, air, soil…) fungi. Afterwards, we removed those OTUs with a representation <0.1% in each sample, as they may be PCR false positives and sequencing errors. This resulted in 510 fungal OTUs (hereafter referred to as 'species'). Given the likely limited quantitative relationship between the fungal DNA sequences obtained and the fungal biomass actually present in Collembola guts (Lamb et al., 2019), we discarded the relative number of sequences assigned to each OTU and used the presence/absence of each OTU. Thus, we could not quantify consumed fungal species, but their occurrence. For each of the 23 sampling trees, we constructed a qualitative trophic interaction network between Collembola species and fungal species present in their guts using these occurrence data (Figure 5.1). Fungal species were classified in the following functional groups, considering their exploitation of resources: saprotrophs, parasites, mycorrhizal fungi, endophytes and lichenized fungi.

Calculation of interaction metrics

To examine the architecture of the Collembola-fungi networks, the following metrics were measured, using the b*ipartite* package (Dormann et al., 2008) in R 3.6.3 (R Core Team, 2020): total number of species, number of links, connectance, nestedness, modularity and robustness. Connectance measures the proportion of realized links among the possible ones. Nestedness describes the degree to which the diet of Collembola are subsets of other, more generalist Collembola and it was calculated using the NODF algorithm, based on overlap and decreasing fill (Almeida-Neto et al., 2008). NODF ranges from 0 (non-nested) to 100 (perfectly nested). Modularity calculates the extent to which interaction networks are compartmentalized into modules. It was computed applying a bipartite version of the modularity proposed by Newman (2006), by using the Beckett's algorithm (Beckett, 2016). Modularity values range from 0 (non-compartmentalized) to 1 (maximum compartmentalization). Robustness measures how the removal of fungi induces secondary extinction among Collembola (Burgos et al., 2007; Memmott et al., 2004), by calculating the area under the curve of the number of species being removed against the number of secondary extinctions. It ranges from 0 to 1, with high values indicating lower number of secondary extinctions and more robust communities. We could not incorporate the rewiring effect on robustness because we lacked information on species traits and fungal abundances required to estimate rewiring probabilities (Vizentin-Bugoni et al., 2020). We also analysed how the removal of fungi induces secondary extinction among fungal functional groups diversity (saprotrophic, parasitic, mycorrhizal, endophytic and lichenized fungi). For both types of robustness, we established three possibilities for the deletion of a species: random extinctions, with least abundant species going extinct first and with best connected species going extinct first.

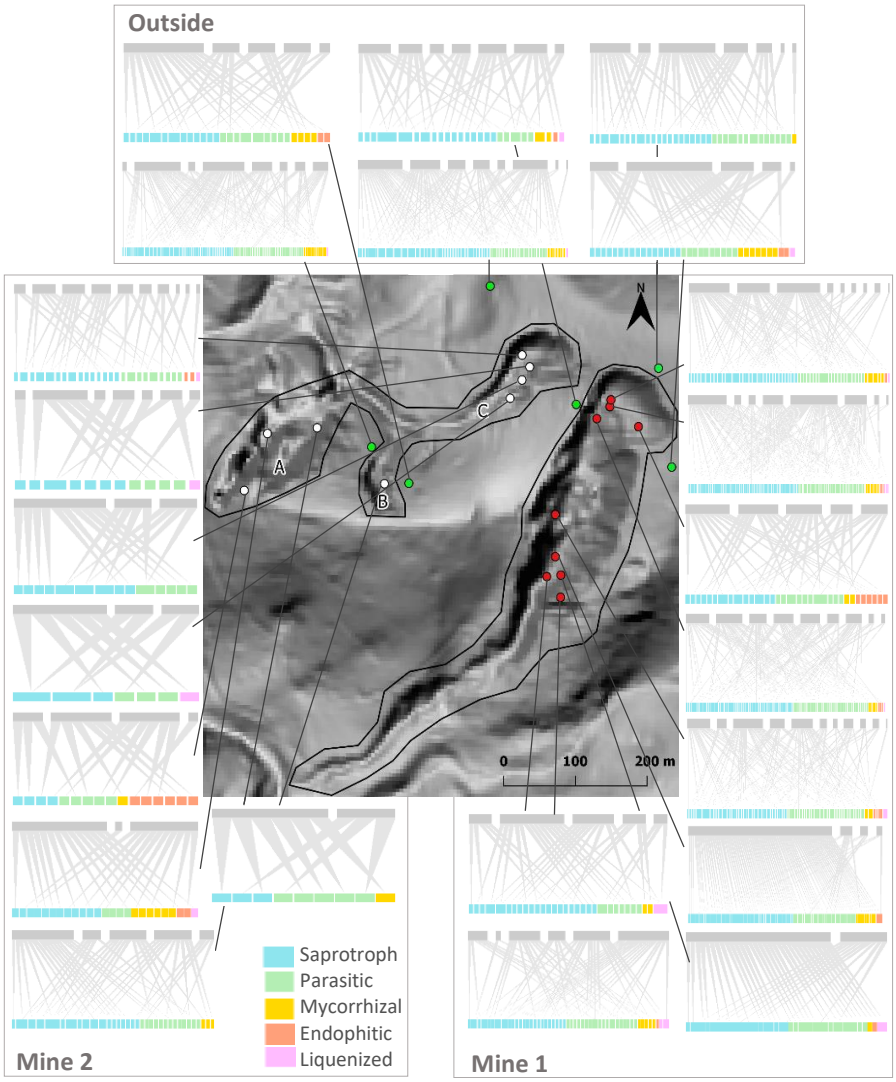

Figure 5.1. Location of the 23 sampling trees inside and outside the mines and their corresponding qualitative trophic networks constructed for Collembola-fungi interactions. Red dots indicate trees inside mine 1; white dots, trees inside cuts A, B and C of mine 2; and green dots, trees outside both mines. Left boxes represent fungal species and right boxes Collembola species. The width of Collembola boxes represents the abundance of interactions.

The deviation of nestedness and modularity from random expectations, was determined by randomization analyses. As nestedness may be associated with connectance (James et al., 2012), we created 100 random networks for each empirical network using the 'swap' algorithm (Miklós & Podani, 2004) from the R package *vegan* (Oksanen et al., 2019), that preserves their species richness and marginal totals (column and row sums in the interaction matrix), and therefore connectance.

Statistical analysis

We examined the recovery of Collembola species richness and diversity (Shannon-Wiener index), by fitting a linear model with location (mine 1, mine 2 and outside) as fixed effect. The species diversity was calculated using the R package *vegan*. For the species richness we fitted a generalized linear model with a Poisson error distribution, using the function *glmer* of R package *lme4* (Bates et al., 2015). We calculated the Bray-Curtis (quantitative) distance index to test for quantitative differences in Collembola communities across locations. We computed a permutational multivariate analysis of variance (PERMANOVA) for Bray-Curtis distances with location as fixed factor, using the *adonis* function of the R package *vegan*, with 999 permutations. To further assess the influence of soil variables on Collembola communities, we run another PERMANOVA analysis for each distance index replacing the effect of location by the effect of all soil variables (C, NH_4, NO_3, P and pH) (see Chapter 4 for differences in soil variables across locations). To visualize the dissimilarity of Collembola communities across locations, a non-metric multidimensional scaling (NMDS) plot was generated using the Bray-Curtis similarity index in the *metaMDS* function of the R package *vegan*. We performed a canonical correspondence analysis (CCA) to visualize the ordination of Collembola communities of mine 1, mine 2 and outside in relation to soil variables.

We analysed the differences in the species richness of fungi that form Collembola diet across locations, following equal model specifications to those of the model for species richness of Collembola communities. We

calculated the Jaccard (binary) similarity index to test for qualitative differences in Collembola-associated fungal community composition across locations. To visualize these differences, we created a NMDS plot for Collembola-associated fungal communities using the Jaccard distance. We looked for associations between the fungal functional groups and the three locations using a Pearson's chi-squared test.

We evaluated the recovery of the architecture of the interaction networks by fitting a linear model for each of the measured network metrics with location as fixed effect. A Kruskal-Wallis test was applied to nestedness model because the assumptions of general linear models (i.e. normal distribution and homogeneity of variances) were not meet by log-transformation. For the total number of species, we used a generalized linear model with a Poisson error distribution.

5.3. Results

Recovery of Collembola community structure

From the 40 Collembola species recorded in total, 70% of their total abundance corresponded to *Isotomiella minor*, *Folsomia manolachei* and *F. minor*, which belong to the family *Isotomidae* (Table S7.1). The species richness of Collembola per tree of mine 1 (6.56 ± 2.88, mean ± s.d.) and mine 2 (4.63 ± 2.67) were not statistically different to the values of the preserved surroundings (6.67 ± 1.37, Table S7.2). The diversity per tree of mine 1 (1.38 ± 0.50) and mine 2 (1.29 ± 0.59) was similar to outside (1.54 ± 0.21, Table S7.2). The PERMANOVA models revealed an effect of location and soil pH on the Collembola communities (Table 5.1). NMDS visualization of Collembola communities was consistent with the effect of location (Figure 5.2). The CCA showed a separation of Collembola communities of mine 2 from mine 1 and outside, that could be explained by the effect of soil pH (Table S7.3; Figure 5.3).

Table 5.1. Results of the PERMANOVA analyses to test the effect of location (mine 1, mine 2 and outside the mines) and soil variables on Collembola communities.

Location					
Source	**Df**	**Sum Sq**	**Pseudo-*F***	**R^2**	***p*(perm)**
Location	2	1.07	1.52	0.13	**0.02**
Residuals	20	7.06		0.88	
Total	22	8.13		1.00	
Soil variables					
Source	**Df**	**Sum Sq**	**Pseudo-*F***	**R^2**	***p*(perm)**
pH	1	0.71	2.02	0.09	**0.003**
NH_4	1	0.49	1.40	0.06	0.13
NO_3	1	0.38	1.09	0.05	0.35
C	1	0.34	0.99	0.04	0.48
P	1	0.27	1.08	0.03	0.75
Residuals	17	5.94		0.73	
Total	22	8.13		1.00	

Df: degrees of freedom; Sum Sq: sum of squares; Pseudo-*F*: *F* value by permutation, *p*(perm): *p*-values based on 999 permutations (statistically significant *p*-values are shown in bold).

Differences in Collembola diets

The most frequently occurring (20% of total presence) fungal taxa in Collembola guts were several species of the order *Helotiales*, *Cordyceps confragosa* and a species of the family *Mycosphaerellaceae* (Table S.8.1). The taxon richness of Collembola-associated fungi per tree of mine 1 (61.56 ± 29.94, mean ± s.d.) were not statistically different to the values of the preserved surroundings (43.83 ± 18.96, Table S8.2), while mine 2 (18 ± 8.97) had significantly lower values than outside (Table S8.2). The PERMANOVA model showed an effect of location on fungal communities (Table 5.2). NMDS visualization of Collembola-associated fungal communities was consistent with the effect of location (Figure 5.4).

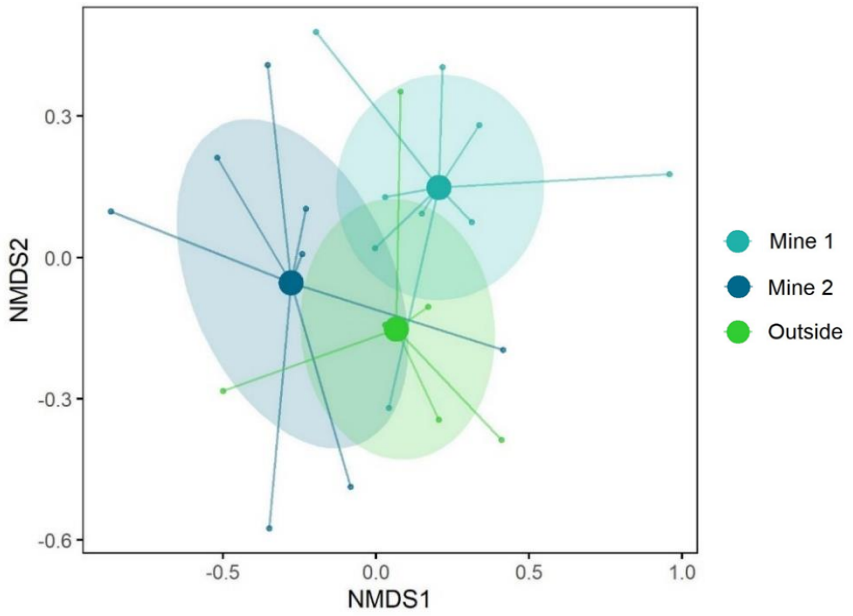

Figure 5.2. Non-metric multidimensional scaling (NMDS) plot of the Collembola communities sampled inside and outside the mines (stress value = 0.19). Points on the ordination space represent sampling trees based on Bray-Curtis similarity index. Centroids and standard deviation ellipses of the three locations are overlaid.

Table 5.2. Results of the PERMANOVA analyses to test the effect of location (mine 1, mine 2 and outside the mines) on Collembola-associated fungal communities in their gut contents.

Source	**Df**	**Sum Sq**	**Pseudo-*F***	**R^2**	***p*(perm)**
Location	2	0.95	1.13	0.10	**0.03**
Residuals	20	8.38		0.90	
Total	22	9.33		1.00	

Df: degrees of freedom; Sum Sq: sum of squares; Pseudo-*F*: *F* value by permutation, *p*(perm): *p*-values based on 999 permutations (statistically significant *p*-values are shown in bold).

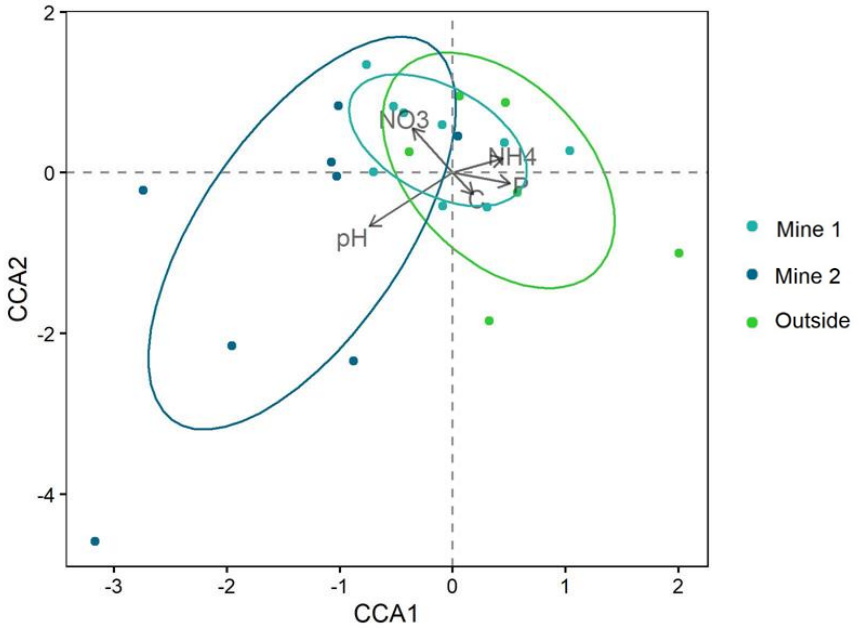

Figure 5.3. Canonical correspondence analysis (CCA) plot of the Collembola communities found inside and outside the mines. Points in the ordination space represent sampling trees based on the effect of the soil variables measured (C, NH_4, NO_3, P and pH). Standard deviation ellipses of the three locations are overlaid.

Overall, saprotrophs represented 55.2 ± 2.1% of Collembola diet, fungal parasites correspond to 31.3 ± 2.4% and mycorrhizal fungi, endophytes and lichenized fungi represent the remaining 7.8 ± 1.9%, 3.6 ± 2.1% and 2.1 ± 0.8% respectively (mean ± s.d. of the three locations together). However, the relative frequencies across locations were slightly different to those expected by the Pearson's chi-squared test (X^2_8 = 13.76, p = 0.088; Figure S2.1). Saprotrophs in guts were more frequent in Collembola outside the mines (57.4%) and slightly less frequent in mine 1 (53.2%). On the contrary, parasites were more frequent in gut of Collembola found in mine 1 (34.0%) than in mine 2 (30.2%) and outside the mines (29.6%). Mycorrhizal fungi were more frequent in Collembola guts outside (9.97%) than inside mine 1 (7.0%) and mine 2 (6.5%). Finally, endophytes were more frequent in mine 2 (5.9%) and lichenized fungi in mine 1 (3.1%), and both groups are less frequent outside (endophytes = 1.8%, lichenized fungi = 1.2%) (Figure 5.5).

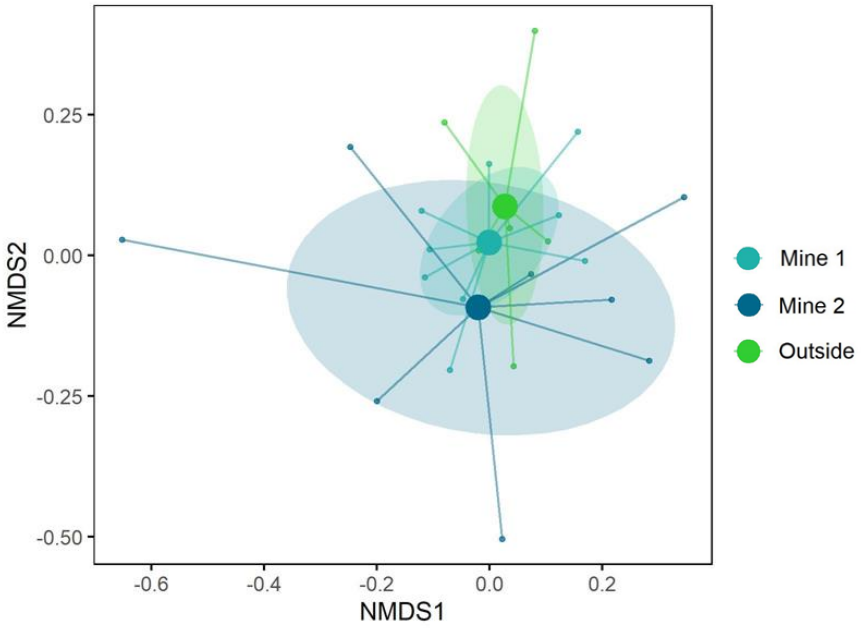

Figure 5.4. Non-metric multidimensional scaling (NMDS) plot of the Collembola-associated fungal communities inside and outside the mines (stress value = 0.21). Points on the ordination space represent sampling trees based on Jaccard similarity index. Centroids and standard deviation ellipses of the three locations are overlaid.

Recovery of Collembola-fungi network architecture

We found similar values between mine 1 and the preserved surroundings for the number of species and the number of links. Conversely, mine 2 had lower values than outside for those two metrics. Connectance was similar between mine 1 and outside, but higher in mine 2 than outside. On average, all networks were slightly nested (mine 1: 15.25 ± 5.04, mine 2: 9.02 ± 14.29, outside: 12.21 ± 7.16, mean ± s.d.). Considering the mean z-score per location to estimate nestedness, networks in mine 1 and outside were not nested, while networks in mine 2 had a lower nestedness than expected by chance. Modularity was lower in both mines than outside, with a higher difference in mine 2. The mean z-score per location for modularity revealed that, on average, networks outside the mines were significantly more modular than the null model, while networks inside were not (Figure 5.6; Table S9.1; Table S9.2).

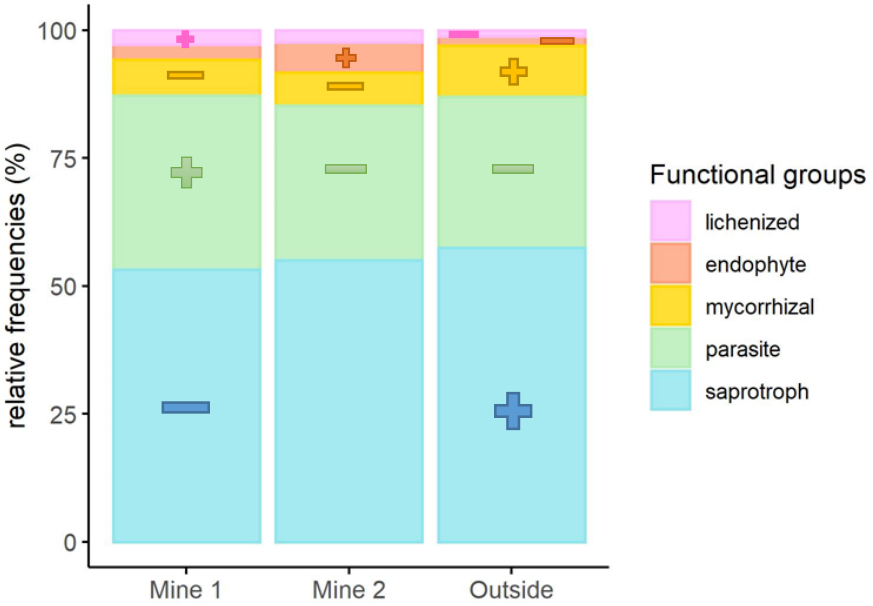

Figure 5.5. Relative frequencies (% of total) of Collembola-associated fungal functional groups (saprotrophs, parasites, mycorrhizal fungi, endophytes and lichenized fungi) inside and outside the mines. Positive signs indicate higher percentages and negative signs indicate lower percentages than expected by the Pearson's chi-squared test.

We did not find strong differences between mine 1 and outside for any of the three types of robustness we used (i.e. least abundant species extinct first, best connected species extinct first or random extinctions). On the other hand, mine 2 robustness differ from outside when both least abundant species and best connected species disappear first. Functional robustness of Collembola-fungal networks was similar inside and outside the mines (Figure 5.7, Table S9.3).

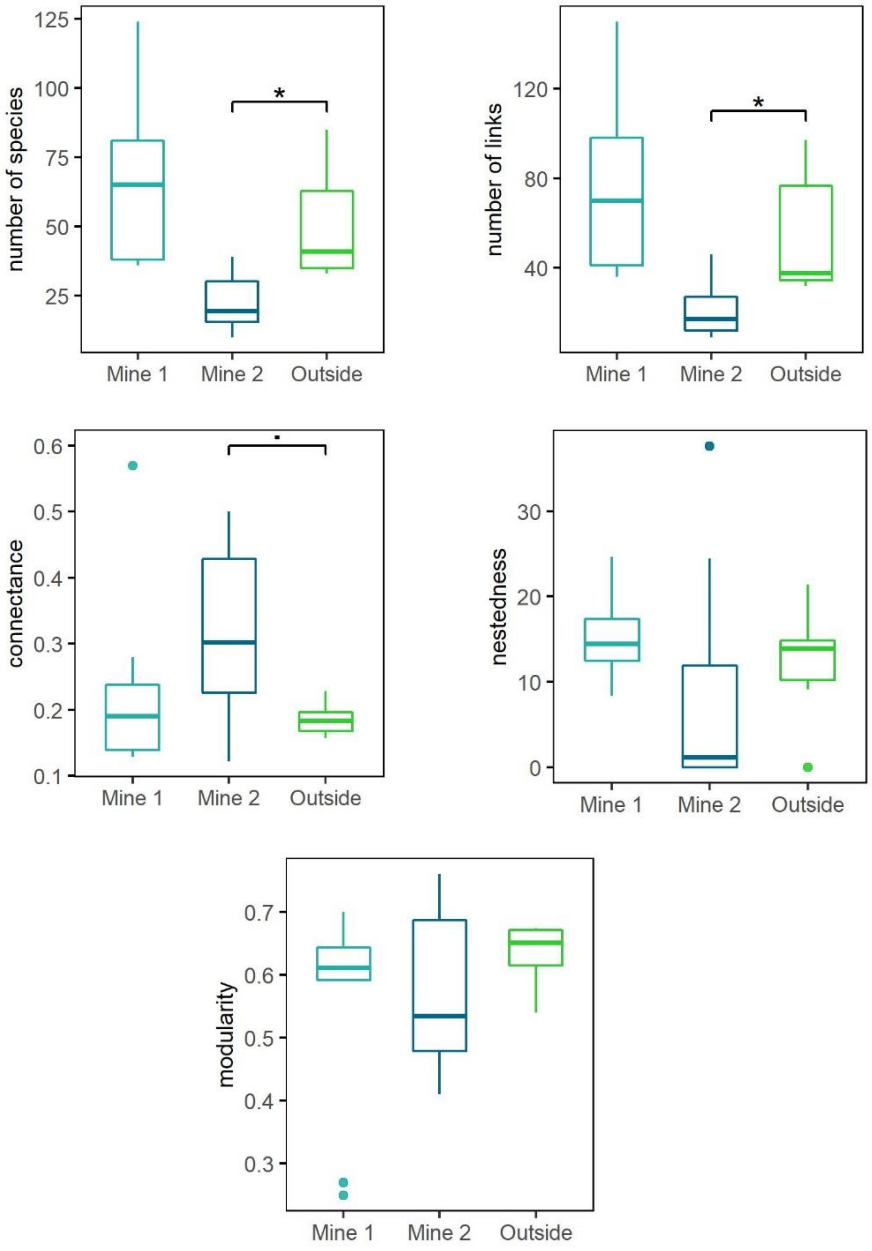

Figure 5.6. Boxplots showing the range of values, inside and outside the mines, for the network metrics number of species, number of links, average degree, connectance, nestedness and modularity. Differences between the mines and the preserved surrounding, as determined by the fitted linear models, are shown with an asterisk (p-value <0.05) or a point (p-value<0.1).

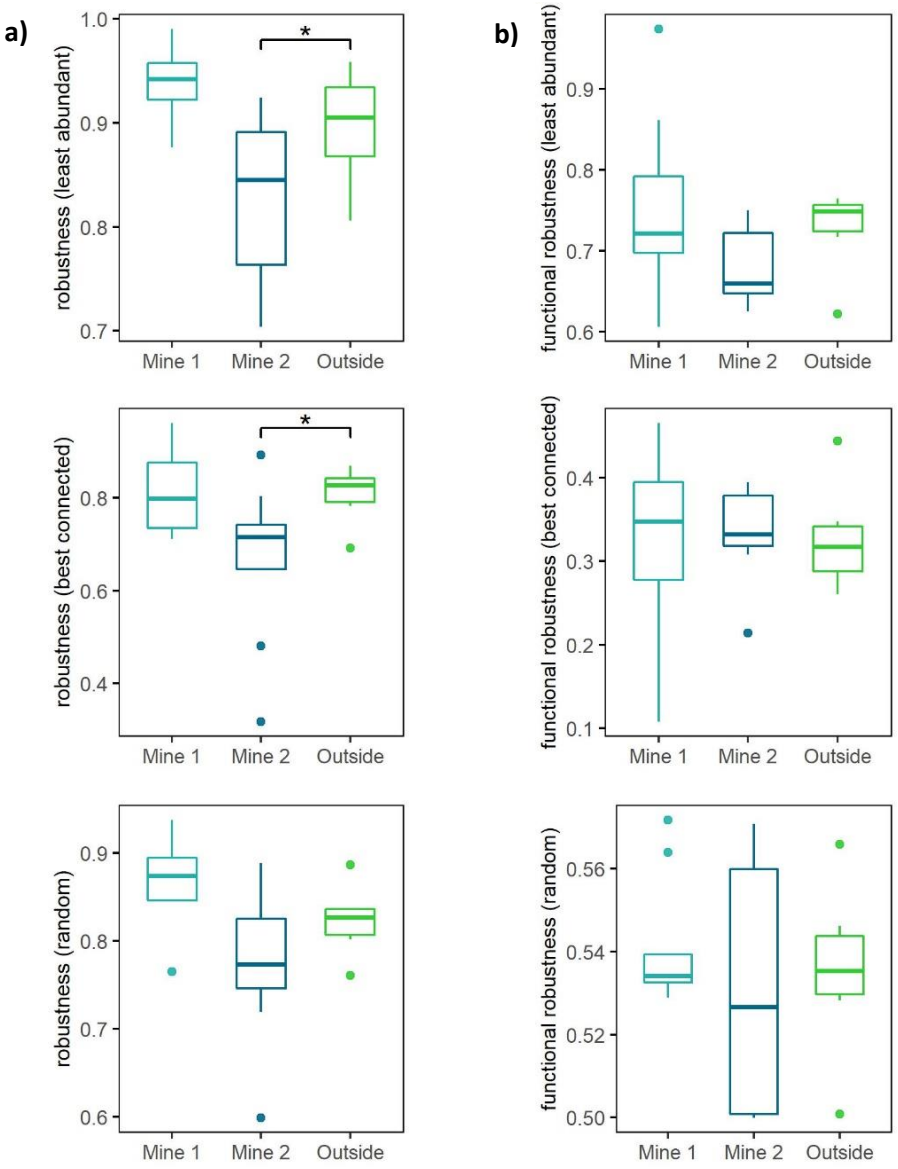

Figure 5.7. Boxplots showing the range of values, inside and outside the mines, for the Collembola robustness to fungal extinctions (a) and the robustness of fungal functional groups (saprotrophs, parasites, mycorrhizal fungi, endophytes and lichenized fungi) to fungal extinctions (b). Three possibilities for species deletion were considered: least abundant species going extinct first, best connected species deleted first or random extinctions. Differences between the mines and the preserved surroundings, as determined by the fitted linear models, are shown with an asterisk (p-value <0.05).

5.4. Discussion

Our results show that mine 1 and mine 2 have recovered their Collembola species richness and diversity after >107 and >148 years of open cast mining abandonment, respectively. Species composition of both mines remains however different from the preserved surroundings. These results extend recovery time from previous studies in post-mining sites reporting still unrecovered Collembola diversity after 16 years (Zeppelini et al., 2009) and species composition after 50 years (Dunger et al., 2004). In forest recovering from logging, Collembola species composition also requires >130 years to recover (Chauvat et al., 2011; Hasegawa et al., 2006). Our results also suggest that dissimilarities in Collembola community composition across locations may be partially explained by the difference in soil pH values. These findings agree with other studies reporting an effect of pH on Collembola community composition (Cassagne et al., 2003; Pollierer & Scheu, 2017).

A good correlation has been identified in beech forests between Collembola gut contents and the food resources present in the surrounding soil (Ponge, 2000). We found that Collembola diet composition was still different inside and outside the mines, which may indicate that fungal communities inside mines have not yet recovered their pre-disturbance structure. Our results also indicate a Collembola's preference for saprotrophic fungi over other functional guilds, like mycorrhizal fungi, in the three locations, which has also been proposed by other authors (Anslan et al., 2018; Potapov & Tiunov, 2016). Many saprotrophs species belong to the order *Helotiales*, an order with many species with active sporulation at sexual (teleomorphic) or asexual (anamorphic) reproductive stages. These saprotrophic fungi are commonly pioneer species that may invest less into crystal structures or other chemical protection of hyphae, as well as poisonous or bitter secondary metabolites to reduce their palatability for arthropods (Anslan et al., 2018; Böllmann et al., 2010). Our results suggest that Collembola diets outside the mines included more mutualistic and

saprotrophic than parasitic fungi, while the opposite was found inside the mines. This division by fungal functional groups may be incomplete, because some fungal taxonomic clades can include mycorrhizal and endophytic species (Tedersoo et al., 2009; Toju et al., 2014).

The network analysis suggests that the interaction networks between Collembolas and fungi in mine 2 did not yet recovered their complexity after >100 years, in terms of the number of interacting species and the links between them. This may be explained by the difference in mining ending dates between mines: activities ended later in mine 2 (cut A and B ended in 1910 and cut C in 1892 ± 15 years) than in mine 1 (1879 ± 25 years). Given that network size is negatively related to connectance (Allesina & Tang, 2012), we found lower connectance in the interaction networks in mine 1, the older, and outside than in mine 2, the younger. This lower connectance may be partially linked with a higher modularity outside and in the older mine (Thébault & Fontaine, 2010), and with the fact that we found the highest (and non-random) modularity outside the mines. The non-compartmentalized networks inside the mines may be also explained by the presence of more generalist Collembola species compared to outside, especially in the younger mine, that hinder the creation of modules in the communities. This is particularly important, as modular networks may be less vulnerable to variations by retaining the impacts of that change within a single module and minimizing propagation of extinctions through the network (Stouffer & Bascompte, 2011; Thébault & Fontaine, 2010). This could explain why robustness values were the lowest in the younger mine.

The small dissimilarities in fungal functional groups between inside and outside the mines may be hindering the detection of differences in their functional robustness. Given that the algorithm we used to compute robustness and functional robustness does not consider the potential ability of species to 'rewire' (i.e. to switch partners) that may occur after species extinction, our results may be underestimating the real robustness and must be cautiously interpreted. The non-nested structures in the networks of the three locations suggest that

Collembola species may divide their diet preferences to avoid competition (Staniczenko et al., 2013), although the null model used may lead to a conservative identification of significant nestedness due to a high Type II error (i.e. failure to reject a false null hypothesis) (Ulrich & Gotelli, 2007).

To better assess the effect of previous mining activities on Collembola-fungi interactions, it would have helped to include replicate sites from other mined forests. Unfortunately, old mines like these, whose recovery process have remained mostly intact (no other relevant impacts have happened in them) for such a long time, are exceptional in temperate regions. Their uniqueness makes them ideal for studying long-term recovery but hampers the possibility to find other sites with similar environmental conditions and resource exploitation approaches. This limitation was overcome by comparing the observed structural attributes of the constructed trophic networks with 100 random networks that preserve their species richness and connectance.

Overall, our outcomes illustrate that below-ground trophic networks may require >150 years to recover their species composition and architecture. Undisturbed forests own unique species and compartmentalized and weakly connected architectures that reduce their vulnerability to extinctions. Evaluating modularity patterns in recovering below-ground communities may provide insights about their recovery process. Actions to accelerate the time that ecosystems require to recover should include restoring species with key effects of network modularity. Further studies should identify those species that act as module connectors (which link different modules) or as hubs (which are highly linked within their module) and that could assist the rebuilding of the architecture below-ground trophic networks. The assessment of restoration performance should focus on the reestablishment of the architecture of those interaction networks less resilient to change, along with other key ecosystem attributes, like community structure, to better predict the time required to full recovery and improve the accountability of restoration actions.

Chapter 6
General discussion

This thesis addresses the limited understanding we have of the forest recovery process over the long-term (>100 years) by applying different methodologies and approaches at global and local scales. Chapter 2 presented a perspective on innovative approaches to address the lack of understanding on long-term ecosystem recovery along with three other constraints of forest restoration success. Chapter 3 assessed the long-term (*c*. 300 years) recovery trajectories of forests worldwide. Chapters 4 and 5 evaluated the recovery of two open mines abandoned for >100 years by focusing on below-ground interactions between trees and ectomycorrhizal (EcM) fungi and between Collembola and soil fungi. The three research chapters used different recovery metrics varying in the amount of ecological information included to test if those metrics that better incorporate ecosystem complexity will better estimate time to recovery.

The three research chapters advance our knowledge about long-term forest recovery by identifying: (1) some of the main drivers regulating this process; (2) better metrics to assess recovery and restoration performance; (3) changes in the shape of the recovery trajectory of the different metrics; and (4) the time required for ecosystem to recover, at least part of their complexity. These main findings are discussed and integrated below, as well as their contribution to improve ecological restoration strategies and outcomes. We also propose future research lines that can be potentially derived from this investigation.

6.1. Long-term forest recovery is a multifactorial process

The type of disturbance has been identified as one main factors affecting forest recovery (Jones et al., 2018; Meli et al., 2017; Moreno-Mateos et al., 2017). Past land use intensity may have strong legacy effects on worldwide forest recovery rate and success (Crouzeilles et al., 2016). However, we found that post-agricultural forests recover faster over the long-term than post-logging forests, which usually implies less intensive impacts (Figure 3.2; Table S3.1; Chapter 3). This was partially explained by the fact that a higher nitrogen availability following agriculture may

favour the recovery of organism abundance and biomass (see *Discussion* in Chapter 3; Du et al., 2020; LeBauer & Treseder, 2008). Ammonium availability was also identified as a driver of EcM fungal community recovery after mining (Figure 4.5; Chapter 4). These novel explanations on the effects of the disturbance type on ecosystem recovery are likely a consequence of the unusually long timescale used in this thesis, which is not commonly found in other recovery studies (Jones & Schmitz, 2009; Martin et al., 2013).

The duration of prior disturbance may also hinder forest recovery (Holl, 2007), but this effect has been poorly studied due to the scarcity of areas disturbed in ancient times (>100–1,000 years) that have not been degraded afterwards, especially in temperate regions (Moreno-Mateos et al., 2020). In this thesis, we faced this constraint by assessing the recovery of a beech forest after more than six centuries of open cast mining operations (chapters 4 and 5). This long period of intensive human disturbance caused severe soil degradation not yet re-stablished after >100 years of recovery affecting soil microbial communities. The legacy of mining operations was the main cause of increased soil pH and decreased ammonium (Carrino-Kyker et al., 2016; Oliveira Silva et al., 2018), which influenced the community composition of beech associated EcM fungi (Table 4.1; Figure 4.5; Goldmann et al., 2015; Tedersoo et al., 2020). The dissimilarities in Collembola community composition were also partially explained by the effect of pH (Table 5.1; Figure 5.3; Cassagne et al., 2003; Pollierer & Scheu, 2017). These results highlight the long-term effects of altered abiotic conditions on the recovery process. Overall, forest recovery is mainly determined by the previous disturbance type, its intensity and duration, as well as the resulting abiotic conditions.

6.2. More complex recovery metrics to better assess restoration success

Ecological restoration has traditionally focused on assessing ecosystem recovery by considering simple attributes (e.g. species diversity), or single functions (e.g. soil organic carbon content) (Holl, 2017). In this thesis, we critically assess the different outcomes derived from recovery metrics varying in the ecological information they include. After evaluating worldwide forest recovery by their organism abundance, species diversity and species composition (Chapter 3), we obtained that after 150 years, forests are closer to reference values of abundance and diversity but still considerably differ in their species composition (Figure 3.2). When we explored this effect at a local scale (chapters 4 and 5), we arrived to similar results. Temperate forests under recovery for more than 140 years after mining operations have already recovered their Collembola community species richness and diversity, but not their species composition (Table 5.1; Figure 5.2; Rozendaal et al., 2019).

The analysis of biotic interactions incorporates more complexity, as interactions consolidation requires not only the presence of certain species but the fulfilment of specific conditions for their encounter (Morales-Castilla et al., 2015). In chapter 4, the interactions between beech trees and EcM fungi, revealed that richness, diversity and taxonomic diversity of mycorrhizal fungal interactions recovered to undisturbed values, whereas interaction identity was still different (Figure 4.3; Table S5.3). Incomplete recovery was also detected when we characterized the architecture of Collembola-fungi networks, suggesting that disturbed areas are still less robust to extinctions than reference, undisturbed areas (Figure 5.6; Figure 5.7).

Recovery was also measured by categorizing EcM fungal traits (i.e. fungal exploration and sporocarp types; Chapter 4) and functional groups of Collembola-associated fungi (i.e. saprotrophs, parasites, mycorrhizal fungi, endophytes and lichenized fungi; Chapter 5). These metrics may be more informative for monitoring purposes than the number of species regardless of their biology (Mazón et al., 2019).

However, this compartmentalization by functional groups may be incomplete in real ecological communities, especially for fungal organisms, as one taxonomic clade can entail more than one functional group and their biology or ecology should be better known (see *Discussion* in Chapter 5; Toju et al., 2014).

Consequently, all these findings suggest that while forests ecosystems may seem recovered over the long term based on metrics with low levels of ecological information, like those measuring the number and abundance of species, complete recovery may not be attained. Metrics about the recovery of biotic interactions including the interaction identity and the network architecture, incorporate more ecological information and might better incorporate the re-assemblage of ecosystem complexity (Moreno-Mateos et al., 2020; Rydgren et al., 2020).

6.3. Shape matters: the decelerating long-term recovery of forests

The pattern and rate of change over time of the ecosystem trajectories towards the reference may exhibit great variation depending on several factors, such as the ecosystem type, the recovery measure or the initial degradation intensity (Bullock et al., 2011). Previous studies have approached the recovery trajectories of forest ecosystems by assuming linear (Curran et al., 2014; Dunn, 2004; Meli et al., 2017) or logarithmic trajectories (F. Martin et al., 2016; Spake et al., 2015). However, they are generally limited to a certain biome or recovery metric or limited a few decades since impact cessation. In Chapter 3, we improved the characterization of recovery trajectories by including multiple recovery metrics of forests worldwide over centuries (*c*. 300 years). But the most innovative progress was the synthesis of 104 chronosequences that included at least two measurements of the condition of the restored ecosystem through time, instead of only the latest condition. We also tested linear and decelerating (logarithmic and root squared) relations

between time since recovery started and the measures of the extent of recovery (see *Methods* in Chapter 3).

The recovery over time of organism abundance, species diversity and nitrogen stock typically follow a logarithmic pattern, carbon cycling and phosphorus stock follow square root recovery trends and species similarity seem to follow a linear shape (Figure 3.2). The rate at which these metrics approached reference values (i.e. the slope of the curve) was higher for organism abundance, carbon cycling and nitrogen stock after agricultural land uses than after logging activities (Table S3.1). The rate of recovery is likely to be lower in less productive ecosystems or if certain abiotic factors constrain recovery (Bullock et al., 2011). In this case, the lower nitrogen availability in post-logging areas, given the common nitrogen limitation of most temperate and tropical forests (Du et al., 2020), may be limiting the recovery of their organism abundance and biomass (see point 6.2).

Overall, forest worldwide commonly follow a decelerating recovery trend. Their recovery rates decrease with time since the disturbance ended, highlighting the crucial role that the first decades of recovery play on ecosystem restoration and the difficulty to reach the final stages of recovery (Jones et al., 2018).

6.4. Forest recovery requires centuries

As suggested above, restored forests might not reach their pre-disturbance state within decadal or centennial timescales (Cole et al., 2014a; Curran et al., 2014). Further results from the three research chapters of this thesis support this statement. Chapter 3 revealed a limited overlap in ecosystem structure and functions between worldwide restored and undisturbed forests after at least 50 years of recovery. Our predictions estimated that the recovery of >90% of reference values of organism abundance, species similarity, carbon cycling and phosphorus stocks require more than two centuries and

more than four centuries for the recovery of species diversity and nitrogen stocks (Table 3.8).

In chapters 4 and 5, we provided deeper insights about the time to recovery of more complex metrics (see point 6.2). The recovery of crucial forest interactions, like the symbiosis between trees and EcM fungi or the trophic interaction between Collembola and soil fungi, was still incomplete after >140 years (see *Discussion* in chapters 4 and 5). The soil fungal species and Collembola species present at late stages of recovery and in old-growth forests may require centuries to appear and even more time may be required for them to interact and build stable networks (Montoya et al., 2015; Morriën et al., 2017).

Given the long periods estimated for the recovery of forest biodiversity, functions and below-ground interactions and considering that forests will require more time to recover even more complex attributes (i.e. above- and below-ground organism interactions) (Moreno-Mateos et al., 2020), complete forest recovery may need many centuries or millennia.

6.5. Restoration applications

Based on the main findings of this thesis, several recommendations for improving forest restoration strategies and practices can be proposed:

- Forest restoration should focus on assisting the recovery of the abiotic conditions and the diversity, composition and interactions of above- and below-ground communities. Therefore, the assessment of restoration success should be based on the re-establishment of all these attributes to incorporate the assemblage of ecosystem complexity. This will contribute to better predict time to recovery and provide deeper insights into the real magnitude of ecosystem degradation.

- Specific measures may be needed to accelerate the re-establishment of those abiotic conditions that would otherwise recover at very large timescales. Soil fertilization can assist

forest recovery after logging activities, considering the low availability of nitrogen and the scarce possibilities of external inputs of this nutrient over time.

- Forest restoration efforts should favour below-ground keystone species existing in late-successional and old-growth forest species that do not readily recolonize to accelerate forest recovery. These actions may imply the introduction of those species or group of species and/or setting the biotic and abiotic conditions they need survive. Soil fungi, and specially, EcM fungi should be prioritized, for their essential role on the recovery of forest ecosystem structure and functioning.

- Actions to accelerate time to forest recovery should also include the restoration of the architecture and deriving stability of below-ground networks. Undisturbed forests present compartmented below-ground networks that promote their robustness to extinctions. Restoration actions should be oriented to re-establish those species that are essential for rebuilding mature network architectures by linking different modules (connectors) or by highly linking their module (hubs).

- Conservation policies should be prioritized protection over restoration. Our results showed that restored forests cannot replace old-growth forests even at long timescales. Among other benefits, old-growth forests harbour many mature-forest specialists, making them an irreplaceable biodiversity resource. In the last years, biodiversity offsetting strategies have been promoted, allowing for forest degradation if compensated through eventual restoration. We recommend caution in promoting restoration to reverse biodiversity and functional loss of forests in detriment of protection as this will mean an increase of less-functional and diverse forest ecosystems. Hence, protective policies in ecosystem management should be prioritised to effectively revert degradation.

- Forest restoration must be planned accordingly to the real timescales at which they actually operate. This thesis evidenced the limitations of restoration and the consequently long time frames for forest recovery. This is particularly critical at this moment that an increasing number of global restoration strategies are being implemented. They set ambitious restoration targets assuming that recovery operates at decadal scales, which would likely imply a limited forest restoration performance and subsequent centennial reductions of biodiversity and ecosystem functions and services. Therefore, if these restoration strategies are planned following centennial timescales, we will set a more feasible scenario to truly restore forests.

6.6. Future research lines

The outcomes of these thesis open opportunities to develop the following research lines:

- Investigate the spatial context-dependency of forest recovery over the long-term. Apart from the abiotic conditions and the previous disturbance type, it is important to further assess the effect of the surrounding landscape on the forest recovery rate and success at ecological time scales (Montoya et al., 2012). As it has been already reported for shorter recovery periods (Crouzeilles et al., 2016), long-term forest recovery may be driven by the landscape context, for instance in less fragmented landscapes (Holl & Aide, 2011). GIS analysis can provide useful information about habitat loss and fragmentation at the landscape scale.

- Assess long-term forest restoration success accounting for what has been already restored. In this thesis we studied the extent of recovery of worldwide forest over the long-term by considering the remaining recovery. This approach could be complemented

with an estimation of the 'Achieved Restoration' index (AR), i.e. what has been done relative to initial degradation intensity (Marchand et al., 2021). The characterization of initial degradation is crucial to inform restoration practitioners and better evaluate restoration outcomes. However, it is important to highlight that the information about the initial disturbed state is not always provided in long-term recovery studies, as it happened in our meta-analysis.

- Replicate the experiments of long-term recovery of below-ground interactions in other places. It is a commonplace that restoration studies lack replicated datasets, especially at long timescales (Ribeiro da Silva et al., 2015). This is mainly due to a uniqueness of forests like Artikutza, under recovery for many decades or centuries that have not been degraded afterwards. One solution to enhance the feasible replicate sites could be to look for long-term recovering forests not necessarily under similar environmental conditions or affected by the same type of disturbance, although these factors could hide the effect of other processes.

- Quantify the intensity of tree-EcM fungi interaction by estimating how much nitrogen is absorbed by the plant thanks to each EcM fungal species (Pena et al., 2013). This will allow to identify highly functional species or clusters of species to be prioritized in forest restoration actions (Hawkins et al., 2015).

- Test when to introduce late successional species that would naturally be re-established at very slow rates. The three research chapters showed that several centuries may be required for old-growth specialists to re-colonized restored forests. Therefore, it is crucial to determine the best moment to reintroduced or favour these species to maximize restoration success.

- Identify which are the most important species for accelerating the recovery of below-ground network architecture. In this thesis we detected that reference forests present more compartmented Collembola-fungi networks, likely because they include certain species that link different modules (connectors) or are highly linked with their module (hubs). Further studies should be oriented in identifying those fungal species so that they can be favoured in restoration practices to rebuild the architecture below-ground trophic networks and their stability.

Chapter 7
Conclusions

1. Based on the meta-analysis, the long-term recovery of worldwide forests increased over time, with faster recovery following agriculture than logging. This was specially the case for organism abundance, carbon cycling and nitrogen stock. Organism abundance recovered faster than species diversity. Woody plants abundance and species similarity reached higher recovery levels than non-woody plants.

2. The time for worldwide forests to recover most of their biodiversity and functions was estimated in more than four centuries.

3. Bases on the observational study, the diversity (i.e. species richness, species diversity and taxonomic diversity) of beech-ectomycorrhizal fungi interactions recovered to undisturbed values after >148 years of mine abandonment, whereas their species identity was still different. *Basidiomycota* and *Ascomycota* abundances and certain fungal functional traits (i.e. exploration and sporocarp types) also reached the values found in undisturbed forests.

4. The signal of old mining operations was partially caused by their long-term impact on soil chemistry. The differences in soil pH and NH_4 showed an effect on the communities of beech associated ectomycorrhizal fungi.

5. The diversity (i.e. species richness and species diversity) of Collembola recovered to undisturbed values after >148 years of mine abandonment, whereas their species composition remained different. As with the ectomycorrhizal fungal communities, this was partly due to the differences in soil pH, derived from mining legacies.

6. Collembola-associated fungal communities inside and outside the mines differed in their functional groups, being mutualistic

and saprotrophic interactions more frequent than parasitic interactions outside the mines.

7. Collembola-soil fungi interaction networks from undisturbed forests presented unique species and compartmentalized and weakly connected architectures that made them more robust to extinctions than networks within the mines.

8. Restoration actions should focus on the reestablishment of the abiotic conditions and the diversity, composition and interactions of above- and below-ground communities to incorporate the assemblage of ecosystem complexity. They should also include measures during the active land use phase to anticipate restoration; identify multiple references; and promote knowledge dissemination outside academia.

9. Global forest restoration strategies and environmental regulations should be planned accordingly to the centennial timescales required for forest recovery.

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Supplementary information of Chapter 3

Appendix S1. Further methodological details.

Study selection

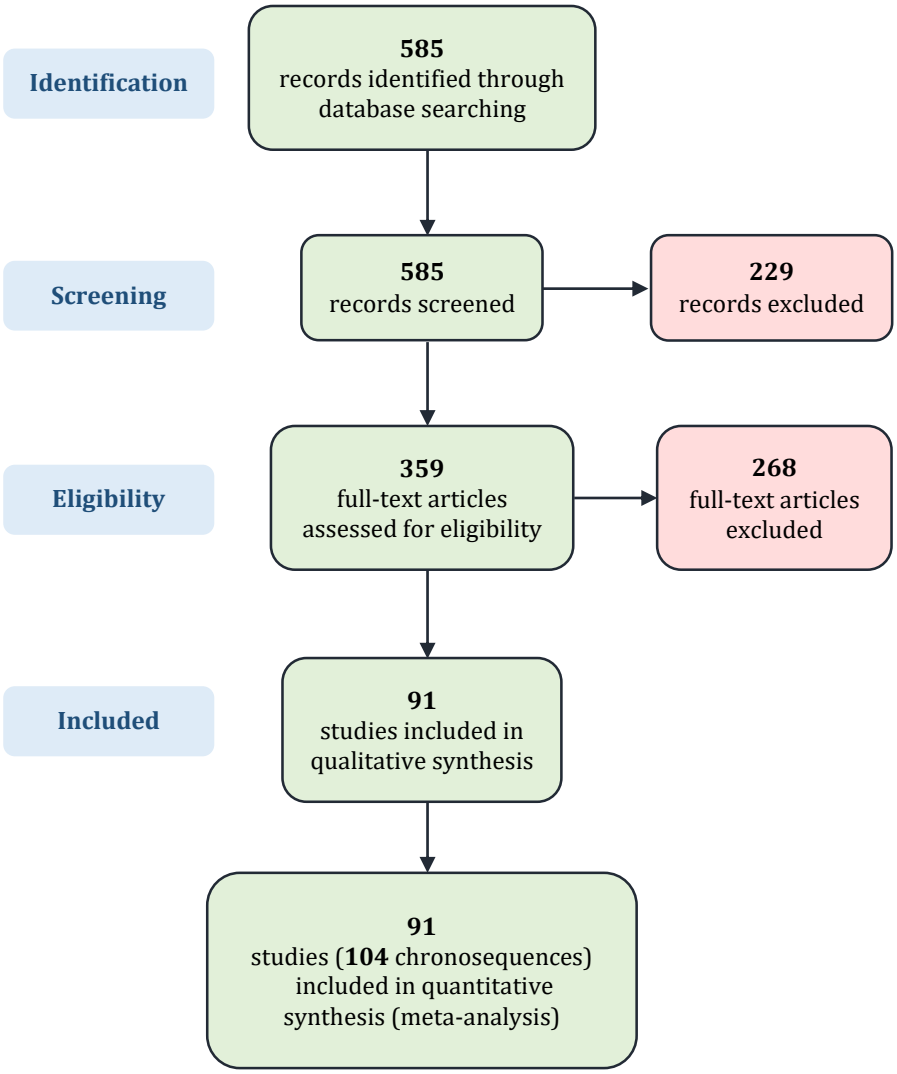

Figure S1.1. PRISMA flow diagram. Structure and template for flow chart from (Moher et al. 2010).

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Database construction

Complementary methods for the extraction of the following variables from the studies:

a) Plot area:

- When outcome measures were obtained by sampling all the trees inside a given a plot or all the trees with a diameter at breast height higher than a given dimension (n = 51, in 7 studies), we assigned a sampled size equal to one.
- When soil cores were extracted and no information was reported about their dimensions (n = 29, in 9 studies), we assumed a diameter of 10 cm, as it was the most common measure in the rest of the cores registered. When a hole was dug and the dimensions were not provided (n = 14, in 5 studies), the assigned dimensions were 30 x 30 cm.
- When transects were used and only the length was provided (n = 12, in 3 studies), we assumed that 1 m was sampled to the right and to the left of that line. Thus, a total width of 2 m.

b) Recovery time:

- When the recovery time was reported as an interval, we considered the average value of that interval. We included those outcome measures (n = 42, in 7 studies) where the maximum average recovery time was below 50 years, but included values of 50 years or more in that interval.
- For those recovery times reported as above or below a certain age, we used the value of that age.

Table S1.1. Distribution of outcome measures, primary studies, chronosequences, data-points, reference sites and study areas by disturbance type, restoration strategy and recovery metric. For the distribution by recovery metric, the sum of the number of studies, chronosequences, data-points, reference sites and study areas is higher than for disturbance type and restoration strategy, because the metric categories are not exclusive

Disturbance	No. outcome measures	No. studies	No. chrono-sequences	No. data-points	Average (min. - max.) no. data-points per chronosequence	Average (min. - max.) time since recovery started	No. reference sites	Area of the study site (km^2)	Studies reporting restored area (%)
Agriculture	340	55	65	569	9 (2 - 39)	82 (50 - 170)	125	105,734	87
Logging	240	36	39	469	12 (2 -54)	96 (50 - 295)	82	51,049	97
Restoration									
Passive	521	84	66	957	10 (2 - 54)	88 (50 - 295)	192	155,013	89
Active	59	7	38	81	0 (3 - 29)	76 (50 - 143)	15	1,770	100
Metric									
Abundance	175	61	70	667	120(3 - 54)	88 (50 - 295)	132	131,315	74
Diversity	148	51	57	572	10 (2 - 39)	83 (50 - 150)	67	23,682	87
Similarity	104	26	29	257	8 (3 - 34)	85 (50 - 150)	36	14,512	78
Carbon	91	45	50	603	12 (2 - 39)	86 (50 - 170)	142	144,081	76
Nitrogen	45	26	26	307	10 (3 - 39)	83 (50 - 170)	46	73,673	81
Phosphorous	17	10	11	169	11 (3 - 39)	68 (50 - 90)	53	3,585	90

Weighting

Random-effect weights were lower for the outcome measures related to biogeochemical recovery metrics (mean; carbon = 9.02, nitrogen = 4.09, phosphorus = 8.84) than for the ones related to abundance (mean = 16.58) or diversity (mean = 31.32). Therefore, we ran a sensitivity analysis using the model for the response ratio and either the random-effect weights or the random-effect weights multiplied by the correction factor $\frac{1}{m_x/\max(m)}$, where m_x is the mean random-effect weight of each recovery metric x and *max(m)* is the maximum mean random-effect weight of all metrics. The parameter estimates were similar, indicating that the model was not sensitive to a corrected random-effect weight.

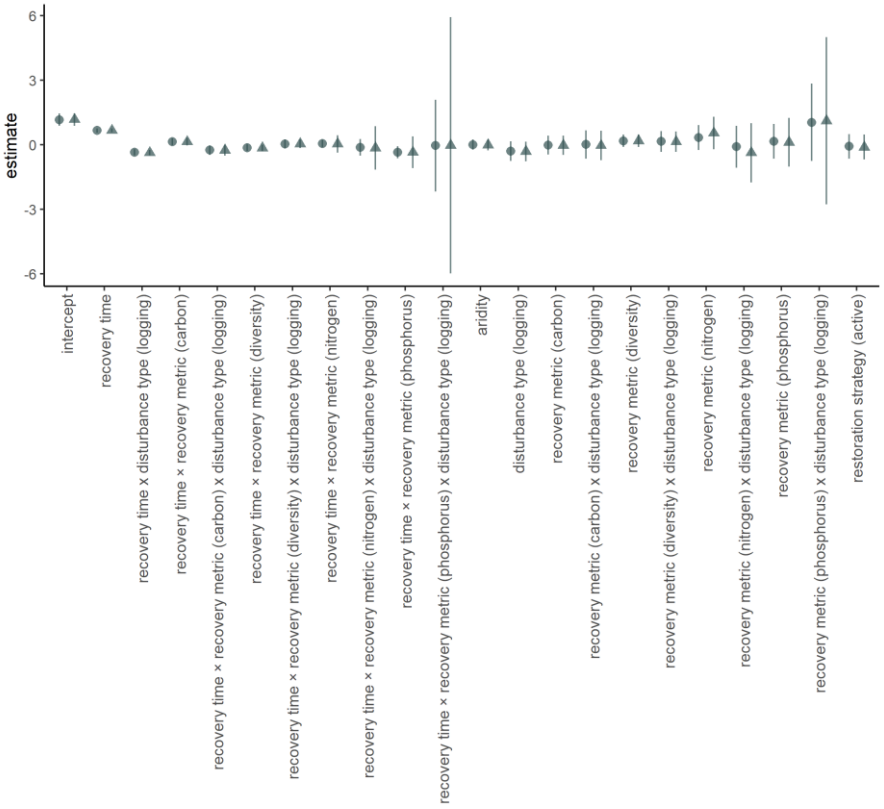


Figure S1.2. Estimated model parameters for the response ratio using the random-effect weights (circles) and the corrected random-effect weights (triangles).

We ran another sensitivity analysis using the model for the rate of change (objective 3) and either the fix-effect weights or the fix-effect weights multiplied by the same correction factor. The parameter estimates were again similar, indicating that the model was not sensitive to a corrected random-effect weight.

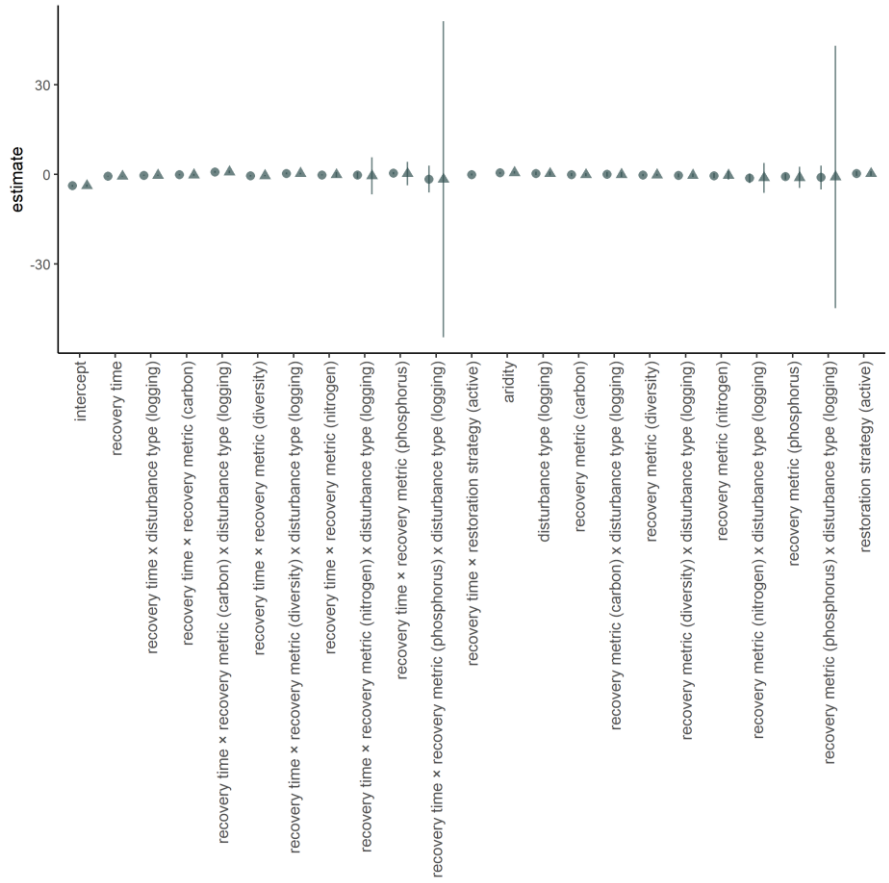


Figure S1.3. Estimated model parameters for the rate of change using the fix-effect weights (circles) and the corrected fix-effect weights (triangles).

Appendix S2. Further details of the statistical analysis

Model for the response ratio (objective 1)

Table S2.1. Model comparisons for the response ratio. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected model is shown in bold. Only the results for the models where ΔAIC ≤ 2 are shown.

Variables							
Recovery time (rt)	**Aridity (a)**	**Disturbance type (dt)**	**Recovery metric (rm)**	**Restoration strategy (rs)**	**Interactions**	**Df**	**AIC**
+		**+**	**+**		**rt × dt + rt × rm**	**15**	**10049.22**
+		+	+	+	rt × dt + rt × rm	16	10051.00
+	+	+	+		rt × dt + rt × rm	16	10051.21

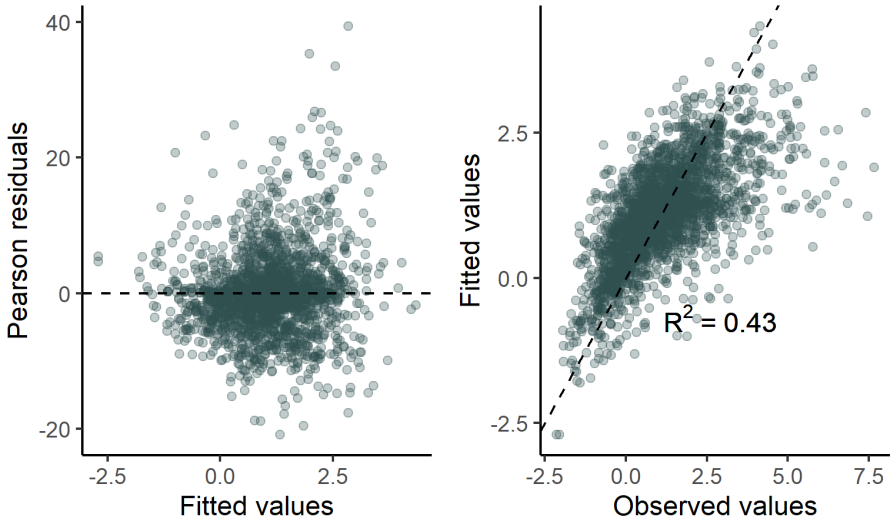


Figure S2.1. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio.

Models for each recovery metric (objective 2)

To calculate the extent of recovery of each metric (objective 2), we fitted separate linear models to each recovery metric. As the effect sizes of similarity variables were calculated differently to the other recovery metrics, we fitted a separate linear mixed model for this metric. First, as we used three different indices to estimate similarity (i.e. Morisita-Horn, Bray-Curtis and Jaccard), we calculated an initial model to discern if different metrics arose in different results. This model included the explanatory variables explained in the main text (i.e. recovery time, disturbance type, aridity and life form), and, in addition, the similarity index. Then, we selected the explanatory variables that better predict the similarity (according to the AIC criterion) and the best model included the similarity index (Table S2.2; Figure S2.2).

The pairwise comparisons among the three similarity indices (Table S2.3) showed that Bray-Curtis index was the only one with no significant differences with the others, so we fitted a separate linear mixed model for this index, which is reported in the main text.

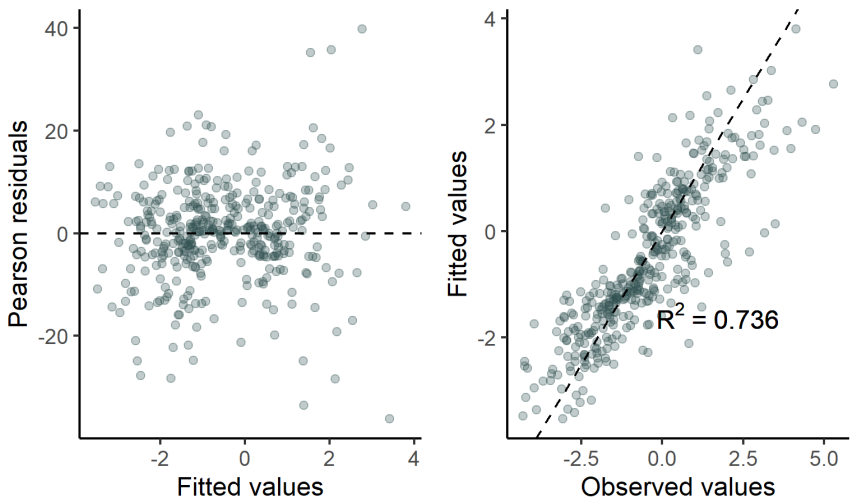


Figure S2.2. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the effect size of similarity.

Table S2.2. Model comparisons for the effect size of similarity. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected model is shown in bold. Only the results for the models where ΔAIC ≤ 2 are shown.

Variables						Df	AIC	ΔAIC
Recovery time (rt)	**Aridity (a)**	**Disturbance type (dt)**	**Life form (lf)**	**Similarity index (rm)**	**Interactions**			
+			**+**	**+**		**11**	**1337.38**	**0**
+	+		+	+		12	1338.51	1.12
+		+	+	+		12	1338.65	1.28

Table S2.3. Pairwise comparisons among the categories of the variable "similarity index" from the model fitted for the effect size of similarity. SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Similarity index	Estimate	SE	*t*-ratio	*p*-value	value ± SE	CI (0.95)
Bray-Curtis					-0.47 ± 0.39	[-1.23, 0.29]
Bray-Curtis – Jaccard	0.30	0.20	1.48	0.30		
Bray-Curtis – Morisita	-0.39	0.20	-1.94	0.13		
Jaccard					-0.77 ± 0.39	[-1.54, -0.01]
Jaccard – Morisita	-0.69	0.20	-3.42	**0.002**		
Morisita					-0.08 ± 0.39	[-0.85, 0.68]

Table S2.4. Model comparisons for the response ratio of each recovery metric. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected models are shown in bold. Only the results for the models where ΔAICc ≤ 2 are shown

Model	Variables						Df	AIC	ΔAIC
	Recovery time (rt)	Aridity (a)	Disturbance type (dt)	Restoration strategy (rs)	Life form (l)	Interactions			
Abundance	**+**		**+**		**+**	**rt × dt**	**12**	**3160.153**	**0**
	+	+	+		+	rt × dt	13	3160.45	0.29
	+		+	+	+	rt × dt	13	3162.15	1.99
Diversity	**+**						**5**	**2418.53**	**0**
	+	+	+			rt × dt	7	2418.66	0.13
	+	+	+			rt × dt	8	2420.02	1.49
	+						6	2420.05	1.52
	+			+			6	2420.34	1.81
	+		+	+		rt × dt	8	2420.43	1.90
	+	+					6	2420.52	1.99
Similarity	**+**				**+**		**9**	**480.40**	**0**
	+	+			+		10	482.01	1.60
	+		+		+		10	482.25	1.84
Carbon	**+**		**+**			**rt × dt**	**7**	**2413.60**	**0**
	+	+	+			rt × dt	8	2414.70	1.10
	+		+	+		rt × dt	8	2415.53	1.93
Nitrogen	**+**		**+**			**rt × dt**	**7**	**958.69**	**0**
	+	+	+			rt × dt	8	958.70	0.02
	+	+	+	+		rt × dt + rt × rs	9	958.84	0.15
	+		+	+		rt × dt + rt × rs	8	959.72	1.03
Phosphorus	**+**	**+**					**6**	**295.62**	**0**
	+		+				6	296.76	1.14
	+	+	+				7	296.99	1.37

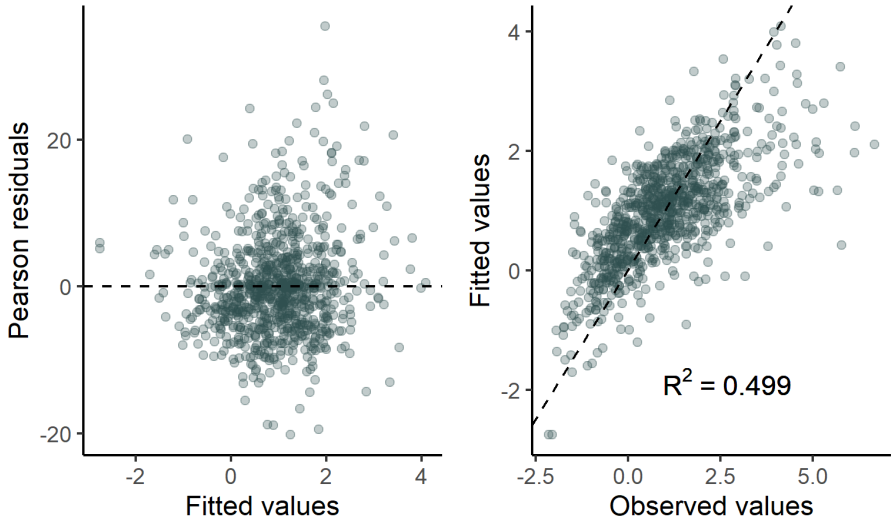


Figure S2.3. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of abundance.

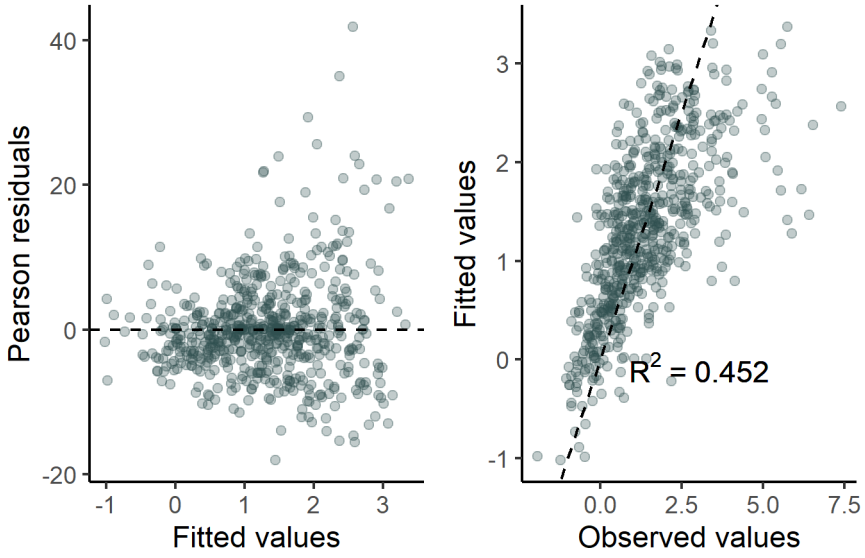


Figure S2.4. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of diversity.

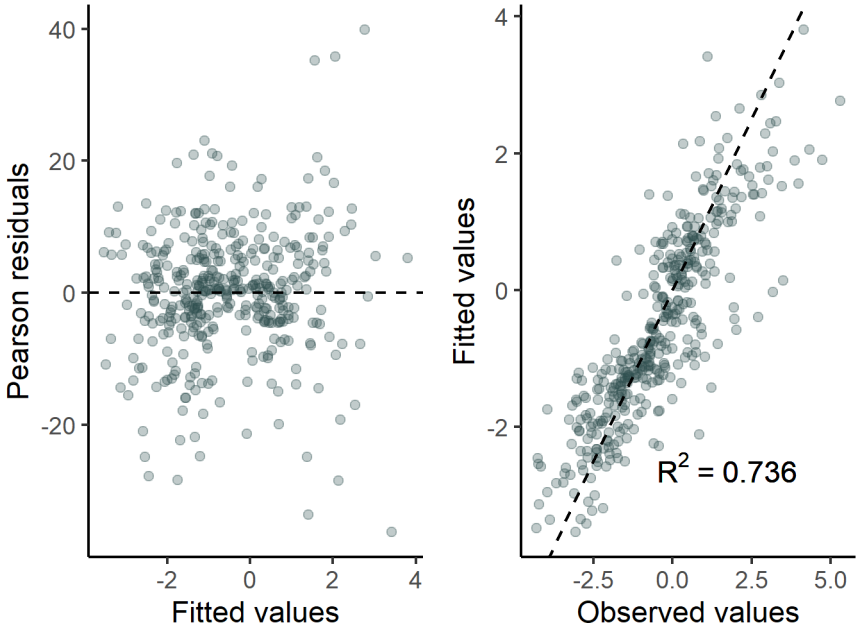


Figure S2.8. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of Bray-Curtis similarity.

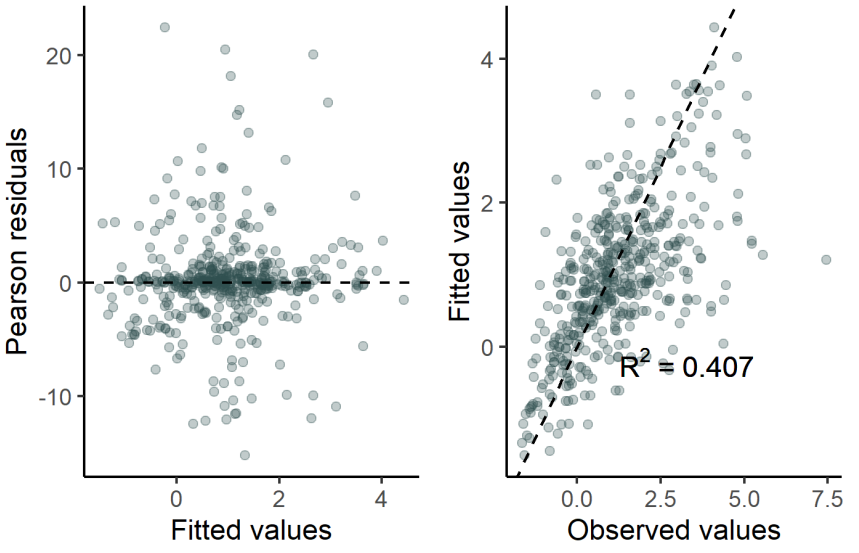


Figure S2.5. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of carbon.

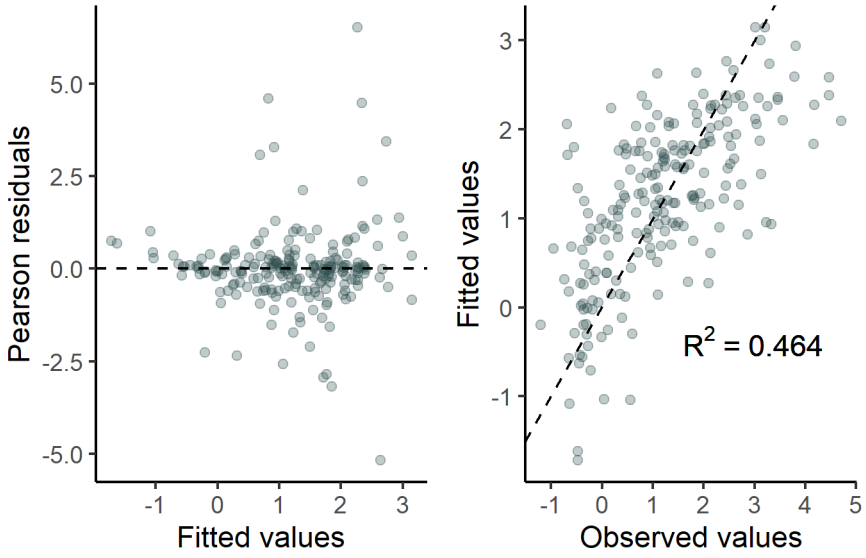


Figure S2.6. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of nitrogen.

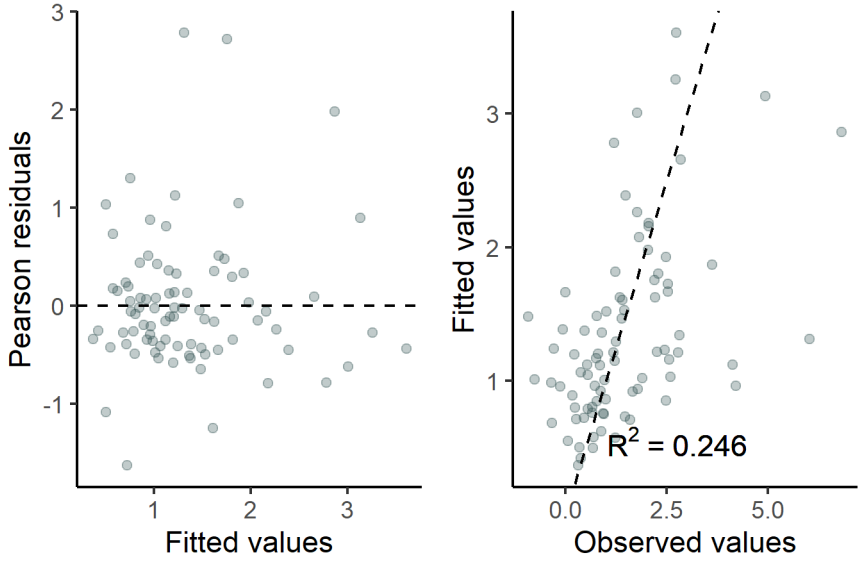


Figure S2.7. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the response ratio of phosphorus.

Models for the rate of change (objective 3)

To estimate the rate of change of the recovery process (objective 3), we fitted a linear mixed model for the rate of change of all recovery metrics. As the effect sizes of similarity variables were calculated differently to the other recovery metrics, we fitted a separate linear mixed model for this metric. First, as we used three different indices to estimate similarity (i.e. Morisita-Horn, Bray-Curtis and Jaccard), we calculated an initial model to discern if different metrics arose in different results. This model included the explanatory variables explained in the main text (i.e. recovery time, disturbance type, aridity and life form), and, in addition, the similarity index. Then, we selected the explanatory variables that better predict the similarity (according to the AIC criterion) and the best model did not include the similarity index (Table S2.5; Figure S2.9). To be consistent with the methodology followed for modelling the effect size of similarity, we fitted a separate model for the rate of change of the Bray-Curtis index, which is reported in the main text.

Table S2.5. Model comparisons for the rate of change of similarity. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected models are shown in bold. Only the results for the models where ΔAIC ≤ 2 are shown.

Variables						Df	AIC	ΔAIC
Recovery time (rt)	**Aridity (a)**	**Disturbance type (dt)**	**Life form (lf)**	**Similarity index (rm)**	**Interactions**			
+		**+**			**rt × dt**	**7**	**1504.31**	**0**
+	+	+			rt × dt	8	1506.03	1.72

Table S2.6. Model comparisons for the rate of change of Bray-Curtis similarity. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected models are shown in bold. Only the results for the models where ΔAIC ≤ 2 are shown.

Variables					Df	AIC	ΔAIC
Recovery time (rt)	**Aridity (a)**	**Disturbance type (dt)**	**Life form (lf)**	**Interactions**			
+		**+**		**rt × dt**	**7**	**552.02**	**0**
+		+			6	552.52	0.50
+	+	+		rt × dt	8	553.68	1.67

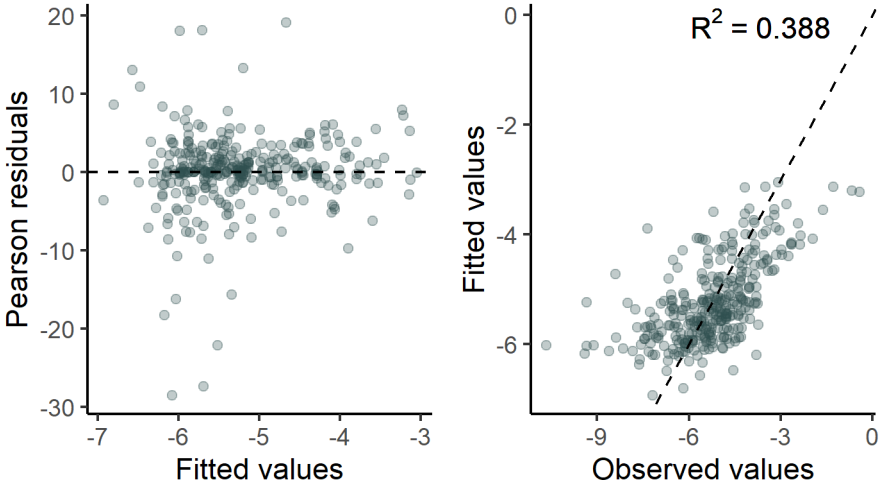


Figure S2.9. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the rate of change of similarity.

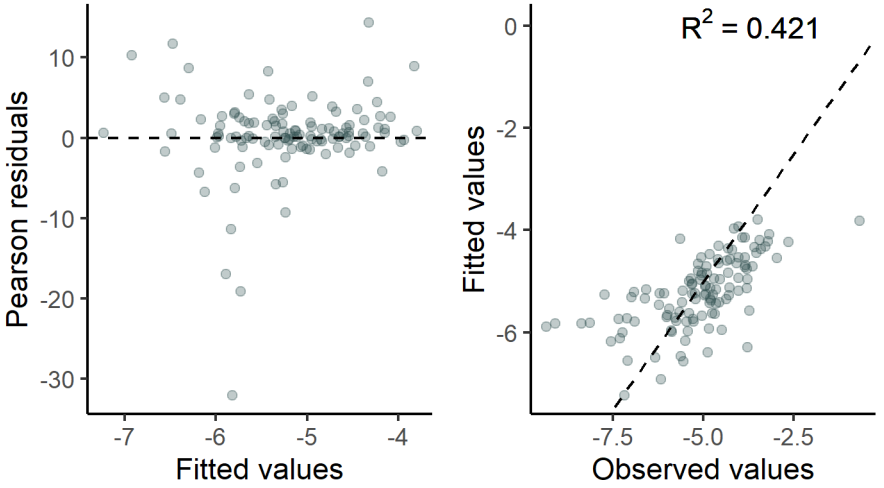


Figure S2.10. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the rate of change of Bray-Curtis similarity.

Table S2.7. Model comparisons for the rate of change. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The selected models are shown in bold. Only the results for the models where ΔAICc ≤ 2 are shown.

Variables						Df	AIC	ΔAIC
Recovery time (rt)	**Aridity (a)**	**Disturbance type (dt)**	**Recovery metric (rm)**	**Restoration strategy (rs)**	**Interactions**			
+	+	+	+		rt × dt + rt × rm + dt × rm + rt × dt × rm	24	12049.51	0
+	**+**		**+**		**rt × rm**	**14**	**12049.96**	**0.45**
+	+	+	+	+	rt × dt + rt × rm + dt × rm + rt × dt × rm	25	12051.15	1.64

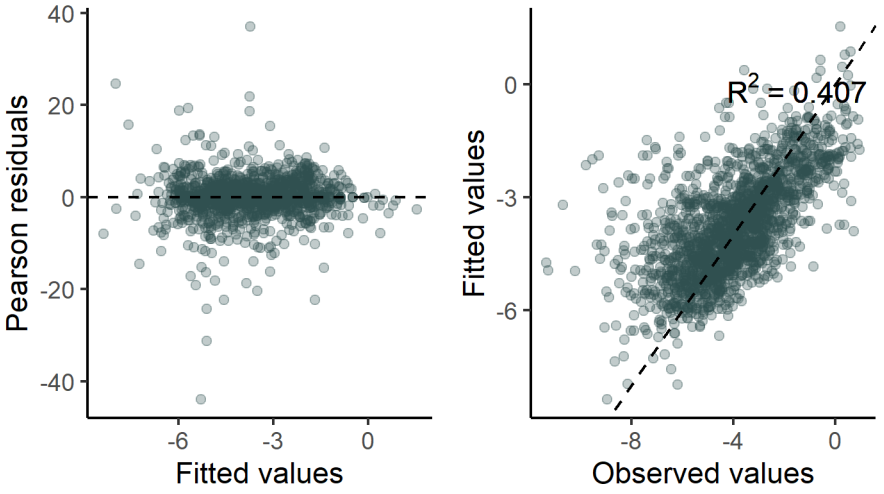


Figure S2.11. Pearson residuals (left) and fitted vs. observed values (right) of the selected model for the rate of change.

Appendix S3. Complementary results

Model for the response ratio (objective 1)

Table S3.1. Results of the model fitted for the response ratio. The response variable of the model equals the inverse of the log-transformed absolute response ratios. Thus, the positive influence of recovery time should be conversely interpreted for forest recovery implications. SE: standard error. Statistically significant *p*-values are shown in bold.

Predictor variable	Estimate	SE	*t*-statistic	*p*-value
Intercept	1.11	0.12	8.88	**<0.001**
Recovery time (ln)	0.73	0.04	18.72	**<0.001**
Recovery metric (carbon)	-0.05	0.17	-0.32	0.75
Recovery metric (diversity)	0.24	0.11	2.12	**0.04**
Recovery metric (nitrogen)	0.46	0.32	1.46	0.15
Recovery metric (phosphorus)	0.22	0.53	0.42	0.68
Disturbance type (logging)	-0.20	0.18	-1.14	0.26
Recovery time × recovery metric (carbon)	-0.04	0.07	-0.65	0.52
Recovery time × recovery metric (diversity)	-0.19	0.05	-3.61	**<0.001**
Recovery time × recovery metric (nitrogen)	0.002	0.18	0.01	0.99
Recovery time × disturbance type (phosphorus)	-0.44	0.30	-1.47	0.15
Recovery time × disturbance type (logging)	-0.31	0.05	-6.26	**0.01**
sd (outcome measure identity)	0.70			
sd (study)	0.59			
AIC	10078.14			

Table S3.2. Pairwise comparisons of recovery time among the categories of the variables "disturbance type" and "recovery metric" from the model fitted for the response ratio. SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Comparisons	Estimate	SE	*t*-ratio	*p*-value	value ± SE	CI (0.95)
Agriculture					0.59 ± 0.07	[0.44, 0.73]
Logging					0.39 ± 0.08	[0.13, 0.44]
Agriculture, Logging	0.31	0.050	6.26	**<0.001**		
Abundance					0.57 ± 0.04	[0.50, 0.64]
Abundance – Carbon	0.04	0.07	0.65	0.97		
Abundance – Diversity	0.19	0.05	3.60	**0.003**		
Abundance – Nitrogen	-0.002	0.18	-0.01	1.00		
Abundance – Phosphorus	0.44	0.30	1.46	0.59		
Diversity					0.38 ± 0.04	[0.31, 0.46]
Diversity – Nitrogen	-0.19	0.18	-1.07	0.82		
Diversity – Phosphorus	0.26	0.31	0.84	0.92		
Carbon					0.53 ± 0.06	[0.42, 0.64]
Carbon – Diversity	0.15	0.07	2.15	0.20		
Carbon – Nitrogen	-0.05	0.18	-0.25	1.00		
Carbon – Phosphorus	0.40	0.31	1.31	0.69		
Nitrogen					0.57 ± 0.17	[0.23, 0.92]
Nitrogen – Phosphorus	0.45	0.35	1.28	0.70		
Phosphorus					0.13 ± 0.30	[-0.47, 0.72]

Models for each recovery metric (objective 2)

Table S3.3. Results of the models fitted for the response ratio of each recovery metric. The response variable of the models equals the inverse of the log-transformed absolute response ratios. Thus, the positive influence of recovery time should be conversely interpreted for forest recovery implications. SE: standard error. Statistically significant *p*-values are shown in bold.

Abundance	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	1.13	0.13	8.66	**<0.001**
Recovery time (ln)	0.71	0.05	14.68	**<0.001**
Disturbance type (logging)	-0.16	0.20	-0.79	0.43
Life form (non-woody)	-1.60	0.45	-3.58	**0.001**
Life form (woody & non-woody)	0.45	0.31	1.44	0.15
Life form (invertebrates)	0.18	0.45	0.40	0.69
Life form (fungi)	-0.05	0.54	-0.09	0.93
Life form (microorganisms)	0.57	0.42	1.36	0.18
Recovery time × disturbance type (logging)	-0.34	0.08	-4.17	**<0.001**
sd (outcome measure identity)	0.71			
sd (study)	0.41			
AIC	3172.33			
Diversity	**Estimate**	**SE**	**t-statistic**	**p-value**
Intercept	1.21	0.14	8.69	**<0.001**
Recovery time (ln)	0.39	0.04	8.89	**<0.001**
sd (outcome measure identity)	0.43			
sd (study)	0.79			
AIC	2425.04			
Similarity (Bray-Curtis)	**Estimate**	**SE**	**t-statistic**	**p-value**
Intercept	-0.83	0.35	-2.35	**0.03**
Recovery time (ln)	0.63	0.09	7.03	**<0.001**
Life form (non-woody)	-2.00	0.60	-3.35	**0.01**
Life form (woody & non-woody)	0.54	0.88	0.61	0.55
Life form (invertebrates)	1.08	0.72	1.49	0.15
Life form (microorganisms)	2.34	1.65	1.41	0.17
sd (outcome measure identity)	0.50			
sd (study)	1.23			
AIC	478.11			

Table S3.3. (cont.)

Carbon	**Estimate**	**SE**	**t-statistic**	**p-value**
Intercept	1.24	0.28	4.38	**<0.001**
Recovery time (√)	0.87	0.06	15.22	**<0.001**
Disturbance type (logging)	-0.24	0.42	-0.56	0.58
Recovery time × disturbance type (logging)	-0.46	0.08	-5.75	**<0.001**
sd (outcome measure identity)	0.58			
sd (study)	0.90			
AIC	2422.66			
Nitrogen	**Estimate**	**SE**	***t*-statistic**	**p-value**
Intercept	1.22	0.20	6.27	**<0.001**
Recovery time (ln)	0.82	0.04	19.71	**<0.001**
Disturbance type (logging)	0.26	0.32	0.81	0.42
Recovery time × disturbance type (logging)	-0.57	0.08	-6.96	**<0.001**
sd (outcome measure identity)	0.72			
sd (study)	0			
AIC	969.11			
Phosphorus	**Estimate**	**SE**	***t*-statistic**	**p-value**
Intercept	1.24	0.22	5.73	**<0.001**
Recovery time (√)	0.25	0.04	7.12	**<0.001**
Aridity	0.54	0.21	2.55	**0.04**
sd (outcome measure identity)	0.57			
sd (study)	0.21			
AIC	303.23			

Table S3.4. Pairwise comparisons of recovery time among the categories of the variable "disturbance type" from the models fitted for the response ratio of "abundance", "carbon" and "nitrogen". SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Comparisons	**Estimate**	**SE**	***t*-ratio**	***p*-value**	**value ± SE**	**CI (0.95)**
Abundance						
Agriculture					0.71 ± 0.05	[0.62, 0.81]
Logging					0.37 ± 0.07	[0.24, 0.50]
Agriculture, Logging	0.34	0.08	4.17	**<0.001**		
Carbon						
Agriculture					0.88 ± 0.06	[0.76, 0.99]
Logging					0.41 ± 0.06	[0.30, 0.52]
Agriculture, Logging	0.46	0.08	5.75	**<0.001**		
Nitrogen						
Agriculture					0.82 ± 0.04	[0.74, 0.90]
Logging					0.25 ± 0.07	[0.11, 0.39]
Agriculture, Logging	0.57	0.08	6.94	**<0.001**		

Table S3.5. Pairwise comparisons among the categories of the variable "life form" from the model fitted for the response ratio of "abundance". SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Abundance					
Comparisons	**Estimate**	**SE**	***t*-ratio**	***p*-value**	**value ± SE**
woody					1.05 ± 0.11
woody – non-woody	1.60	0.46	3.53	**0.006**	
woody – woody & non-woody	-0.45	0.31	-1.44	0.70	
woody – invertebrates	-0.18	0.46	-0.40	1.00	
woody – fungi	0.05	0.54	0.09	1.00	
woody – microorganisms	-0.57	0.42	-1.34	0.76	
non-woody					-0.54 ± 0.44
non-woody – woody & non-woody	-2.04	0.53	-3.86	**0.002**	
non-woody – invertebrates	-1.78	0.62	-2.87	**0.05**	
non-woody – fungi	-1.55	0.69	-2.24	0.22	
non-woody – microorganisms	-2.17	0.61	-3.61	**0.004**	
woody & non-woody					1.50 ± 0.30
woody & non-woody – invertebrates	0.26	0.54	0.49	1.00	
woody & non-woody – fungi	0.50	0.60	0.82	0.97	
woody & non-woody – microorganisms	-1.24	0.50	-0.25	1.00	
invertebrates					1.24 ± 0.44
invertebrates – fungi	0.23	0.69	0.34	1.00	
invertebrates – microorganisms	-0.39	0.60	-0.64	0.99	
fungi					1.00 ± 0.53
fungi, microorganisms	-0.62	0.55	-1.13	0.87	
microorganisms					1.63 ± 0.41

Table S3.6. Pairwise comparisons among the categories of the variable "life form" from the model fitted for the effect size of "Bray-Curtis similarity". SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Bray-Curtis similarity					
Comparisons	**estimate**	**SE**	***t*-ratio**	***p*-value**	**value ± SE**
woody					-0.85 ± 0.36
woody – non-woody	2.00	0.60	3.34	**0.008**	
woody – woody & non-woody	-0.54	0.88	-0.61	0.97	
woody – invertebrates	1.08	0.72	1.48	0.58	
woody – microorganisms	-2.34	1.67	1.40	0.63	
non-woody					-2.85 ± 0.67
non-woody – woody & non-woody	-2.54	1.05	2.43	0.11	
non-woody – invertebrates	3.08	0.92	3.36	**0.007**	
non-woody – microorganisms	-4.34	1.77	2.46	0.10	
woody & non-woody					-0.31± 0.81
woody & non-woody – invertebrates	0.54	1.03	0.53	0.98	
woody & non-woody – microorganisms	-1.80	1.82	0.99	0.86	
invertebrates					0.23 ± 0.63
invertebrates – microorganisms	-1.26	1.75	-0.72	0.95	
microorganisms					1.49 ± 1.64

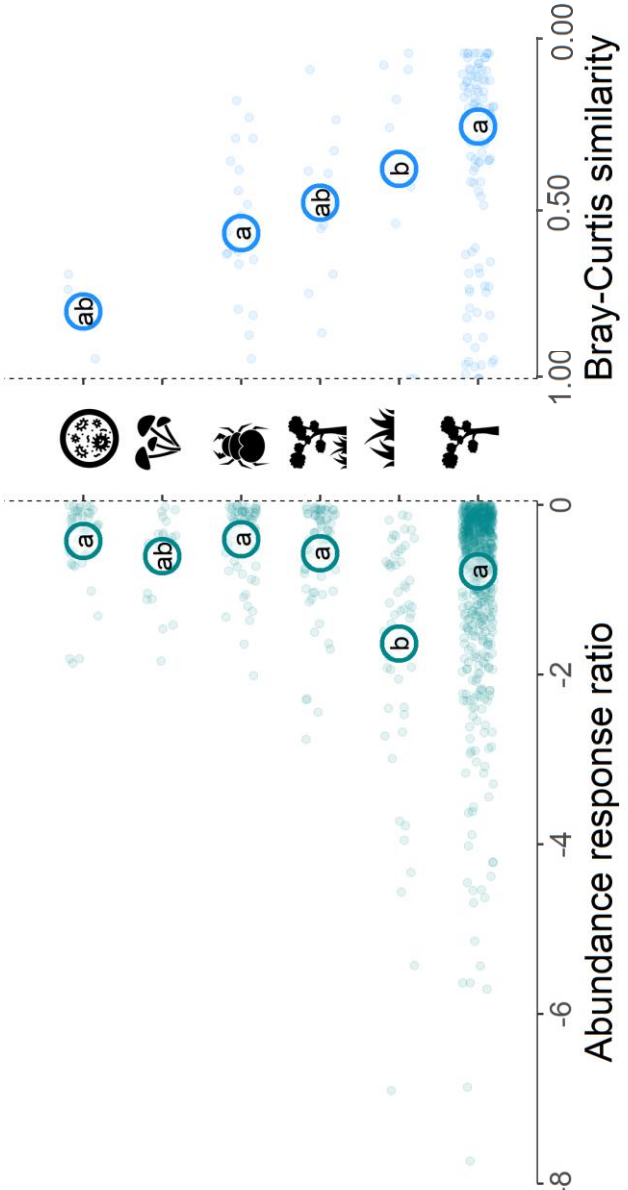


Figure S3.1. Response ratio values for abundance outcome measures (left) and effect size values for similarity outcome measures across life forms. Life form categories not sharing the same lower case letters are significantly different. Circles indicate the median value. Icon source: Jacqueline Fernandes, BomSymbols, Nattawut Buahom, SBTS and Tippawan Sookruay (The Noun Project), accessed through www.thenounproject.com.

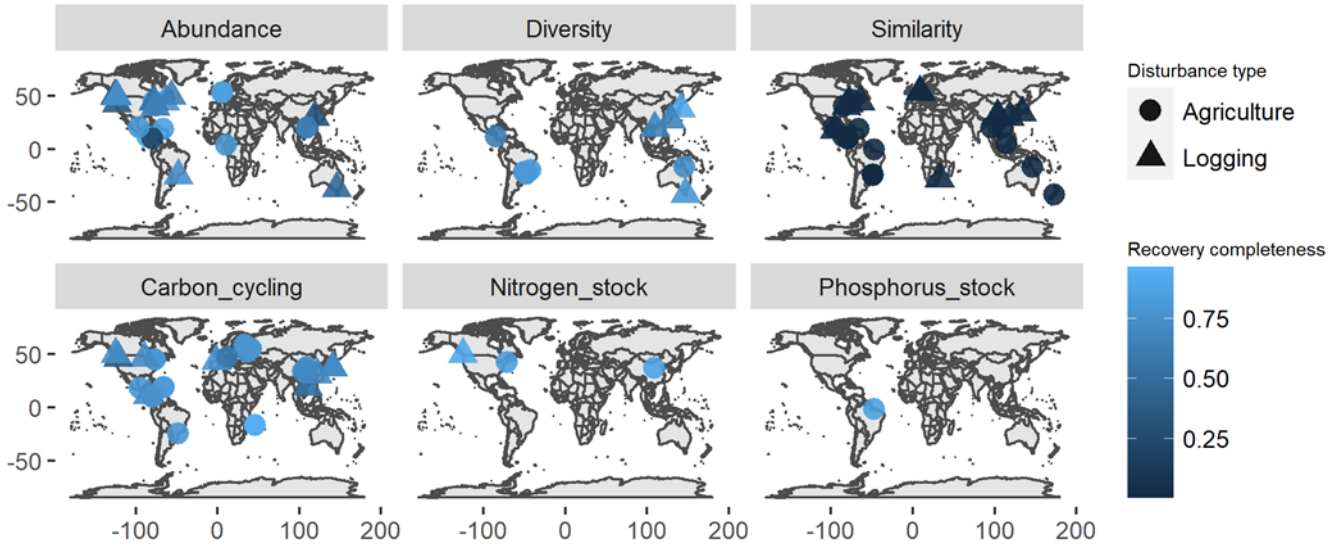


Figure S3.2. Global distribution of the selected chronosequences by the recovery metric with the lowest recovery completeness after 50 years since disturbance cessation. It was calculated on the basis of a model of the response ratio of organism abundance, species diversity, carbon cycling and nitrogen and phosphorus stocks and a model of the effect size of species similarity, following agriculture or logging (see *Methods*).

Time to recovery

Table S3.7. Extent of recovery with respect to reference values at the maximum recovery time found for each recovery metric. CI: Confidence Interval.

Maximum time since recovery started			
Recovery metric	**Recovery time (years)**	**Extent of recovery (%)**	**CI (0.95)**
Agriculture			
Abundance (woody)	143	88.97	[85.46 – 91.68]
Abundance (non-woody)	115	51.13	[20.03 – 75.59]
Logging			
Abundance (woody)	200	82.05	[75.06 – 87.25]
Abundance (non-woody)	295	42.82	[12.58 – 70.68]
Diversity	150	85.74	[81.15 – 89.29]
Similarity (woody)	150	74.25	[55.24– 87.08]
Similarity (non-woody)	100	13.71	[4.05 – 37.39]
Carbon (agriculture)	170	86.82	[76.43 – 92.84]
Carbon (logging)	152	97.04	[94.53 – 98.41]
Nitrogen (agriculture)	170	93.30	[90.08 – 95.50]
Nitrogen (logging)	90	84.47	[75.25 – 90.47]
Phosphorus	90	81.16	[72.46 – 87.98]

Table S3.8. Percentage of recovery with respect to reference values at different times since recovery started: 73, 146 and 219 years of recovery (i.e. one, two and three times the global life expectancy). CI: Confidence Interval.

	73 years		*146 years*		*219 years*	
Recovery metric	**Extent of recovery (%)**	**CI (0.95)**	**Extent of recovery (%)**	**CI (0.95)**	**Extent of recovery (%)**	**CI (0.95)**
Agriculture						
Abundance (woody)	82.97	[78.30 – 86.72]	89.12	[85.64 – 91.80]	91.68	[88.76 – 93.88]
Abundance (non-woody)	39.81	[11.08 – 68.01]	56.67	[25.53 – 78.95]	65.17	[35.53 – 83.76]
Logging						
Abundance (woody)	75.23	[67.43 – 81.42]	80.12	[79.95 – 85.58]	82.58	[75.63 – 87.70]
Abundance (non-woody)	24.57	[35.48 – 55.43]	33.52	[7.25 – 63.42]	38.89	[10.15 – 67.72]
Diversity	81.78	[76.56 – 85.95]	85.61	[81.00 – 89.18]	87.49	[83.18 – 90.76]
Similarity (woody)	42.07	[26.53 – 59.37]	72.86	[53.75 – 86.12]	90.85	[77.01 – 96.71]
Similarity (non-woody)	89.21	[25.94 – 26.49]	26.58	[8.27 – 59.25]	57.23	[21.94 – 86.44]
Carbon (agriculture)	88.02	[79.92 – 93.00]	96.00	[92.76 – 97.80]	98.31	[96.74 – 99.13]
Carbon (logging)	77.89	[63.15 – 87.30]	86.36	[75.73 – 92.56]	90.72	[82.45 – 95.21]
Nitrogen (agriculture)	87.89	[82.61 – 91.65]	92.54	[89.01 – 94.96]	94.40	[91.64 – 96.27]
Nitrogen (logging)	83.79	[74.41 – 89.96]	85.95	[77.03 – 91.59]	87.08	[78.36 – 92.46]
Phosphorus	79.82	[70.13 – 86.67]	85.85	[77.96 – 91.07]	89.31	[82.51 – 93.56]

Table S3.9. Estimated time to reach 90% of reference values for each recovery metric. SE: standard error.

Metric type	Time to recovery (years)	Median	SE
Abundance		126.57	147.79
Abundance –Agriculture			
Abundance (woody)	165.89		
Abundance (non-woody)	1636.92		
Abundance (woody & non-woody)	87.33		
Abundance (invertebrates)	127.63		
Abundance (fungi)	178.24		
Abundance (microorganisms)	73.09		
Abundance –Logging			
Abundance (woody)	146.70		
Abundance (non-woody)	1152.73		
Abundance (woody & non-woody)	61.50		
Abundance (invertebrates)	89.88		
Abundance (fungi)	125.52		
Abundance (microorganisms)	51.47		
Diversity	414.46	414.46	-
Similarity		183.51	39.81
Similarity (woody)	213.55		
Similarity (non-woody)	325.42		
Similarity (woody & non-woody)	183.51		
Similarity (invertebrates)	153.48		
Similarity (microorganisms)	83.00		
Carbon		143.65	60.15
Carbon (agriculture)	83.51		
Carbon (logging)	203.80		
Nitrogen		417.16	320.90
Nitrogen (agriculture)	96.25		
Nitrogen (logging)	738.08		
Phosphorus	196.27	196.27	-

Table S3.10. Results of the fitted model for the rate of change. Higher values of the aridity index mean lower aridity. SE: standard error. Statistically significant *p*-values are shown in bold.

Predictor variable	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	-3.58	0.12	-29.04	<0.001
Recovery time (ln)	-1.27	0.06	-20.82	**<0.001**
Recovery metric (diversity)	-0.39	0.14	-2.78	**0.006**
Recovery metric (carbon)	-0.20	0.21	-0.92	0.35
Recovery metric (nitrogen)	-0.53	0.68	-0.78	0.44
Recovery metric (phosphorus)	-1.26	1.64	-0.77	0.44
Aridity	0.67	0.15	4.61	**<0.001**
Recovery time × recovery metric (diversity)	0.002	0.07	0.03	0.98
Recovery time × recovery metric (carbon)	0.55	0.11	5.03	**<0.001**
Recovery time × recovery metric (nitrogen)	0.13	0.56	0.24	0.81
Recovery time × disturbance type (logging)	0.84	1.57	0.54	0.59
sd (outcome measure identity)	0.64			
sd (study)	0.62			
AIC	12062.42			

Table S3.11. Results of the fitted model for the Bray-Curtis similarity rate of change. SE: standard error. Statistically significant *p*-values are shown in bold.

Predictor variable	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	-5.23	0.18	-29.09	**<0.001**
recovery time (√)	-0.58	0.11	-5.12	**<0.001**
sd (outcome measure identity)	0.54			
sd (study)	0.00			
AIC	558.14			

Table S3.12. Pairwise comparisons of recovery time among the categories of the variables "recovery metric" from the model fitted for the rate of change. SE: standard error. CI: confidence interval. Statistically significant *p*-values are shown in bold.

Comparisons	Estimate	SE	*t*-ratio	*p*-value	value ± SE	CI (0.95)
Abundance					-1.27 ± 0.06	[-1.39, -1.15]
Abundance – Diversity	-0.002	0.07	-0.03	1.00		
Abundance – Carbon	-0.55	0.11	-5.03	**<0.001**		
Abundance – Nitrogen	-0.13	0.54	-0.24	1.00		
Abundance – Phosphorus	-0.84	1.23	-0.68	0.96		
Diversity					-1.27 ± 0.04	[-1.35, -1.18]
Diversity – Carbon	-0.55	0.10	-5.45	**<0.001**		
Diversity – Nitrogen	-0.13	0.54	-0.24	1.00		
Diversity – Phosphorus	-0.84	1.23	-0.68	0.96		
Carbon					-0.72 ± 0.09	[-0.90, -0.54]
Carbon – Nitrogen	0.41	0.56	0.74	0.95		
Carbon – Phosphorus	-0.29	1.23	-0.24	1.00		
Nitrogen					-1.13 ± 0.55	[-2.23, -0.33]
Nitrogen – Phosphorus	-0.70	1.31	-0.54	0.98		
Phosphorus					-0.43 ± 1.23	[-3.13, 2.27]

Supplementary information of Chapter 4

Elaphomyces mutabilis

Appendix S4. Identification of ectomycorrhizal fungi OTUs

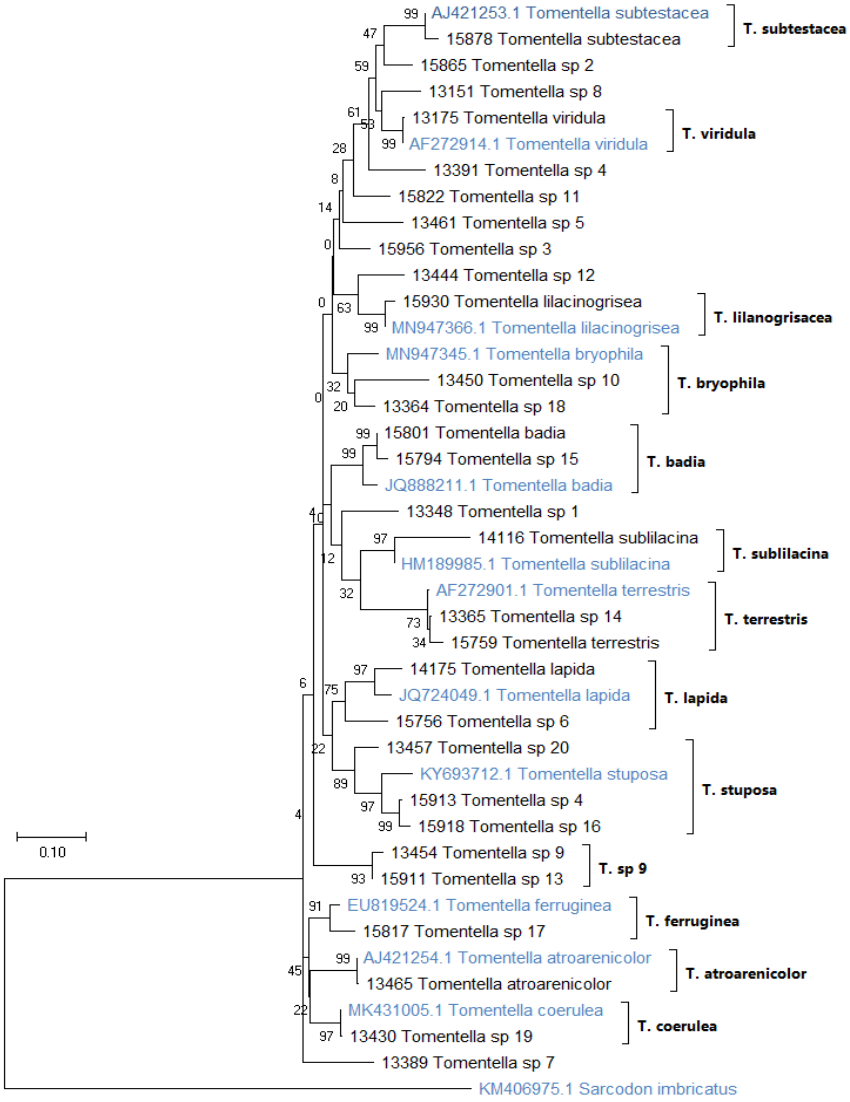


Figure S4.1. Phylogenetic placement of sequences from the genus *Tomentella*, using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980) with a discrete Gamma distribution. The ITS phylogram consists of 13 identified species (in bold) and was built with 42 sequences: 29 sequences from the ectomycorrhizal root tips sampled (each one representing a potential species), and 13 sequences from GenBank (in blue), corresponding to the closest matching sequences and one outgroup. Branch lengths measured the number of substitutions per site.

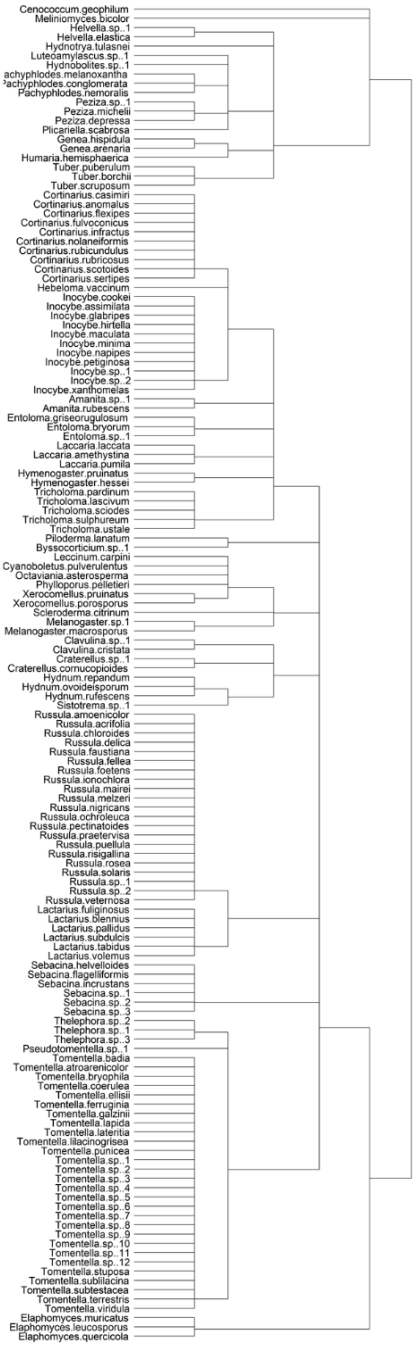


Figure S4.2 Dendrogram of taxonomic distances of the ectomycorrhizal fungal species identified on European beech roots with and outside mines.

Appendix S5. Recovery of interactions and functional traits

Table S5.1. Ectomycorrhizal fungal species identified at 97% DNA similarity level from GenBank data (M1 = mine 1, M2 = mine 2, Out = outside the mines).

Basidiomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	*Amanita* sp. 1	MW282639		X	
Agaricomycetes	Atheliales	Atheliaceae	*Byssocorticium*	*Byssocorticium* sp. 2	MW282459, MW282737		X	X
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius anomalus* (Fr.: Fr.) Fr.	MW282543, MW282613			X
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius casimiri* (Velen.) Huijsman	MW282537	X		
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius flexipes* (Pers.: Fr.) Fr.	MW282704		X	
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius fulvoconicus* M.M. Moser	MW282685	X		
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius infractus* (Pers.: Fr.) Fr.	MW282545		X	
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius rubicundulus* (Rea) A. Pearson	MW282663			X
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius rubricosus* (Fr.) Fr.	MW282380	X		
Agaricomycetes	Agaricales	Cortinariaceae	*Cortinarius*	*Cortinarius decipiens* (Pers.: Fr.) Fr.	MW282350, MW282584			X
Agaricomycetes	Agaricales	Cortinariaceae	*Hebeloma*	*Hebeloma vaccinum* Romagn.	MW282374			X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe assimilata* Britzelm.	MW282400, MW282538			X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	Inocybe cookei Bres.	MW282391, MW282334, MW282377, MW282421 MW282443, MW282455, MW282601		X	X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe glabripes* Ricken	MW282409, MW282480, MW282604 , MW282647		X	X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe maculata* Boud.	MW282390, MW282467, MW282515, MW282554, MW282564, MW282574, MW282589, MW282606, MW282661	X	X	
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe napipes* J.E. Lange	MW282427			X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe petiginosa* (Fr.: Fr.) Gillet	MW282559, MW282703	X		X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe* sp. 1	MW282472, MW282614, MW282698			X
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe* sp. 2	MW282337, MW282364, MW282557	X	X	
Agaricomycetes	Agaricales	Cortinariaceae	*Inocybe*	*Inocybe xanthomelas* Boursier & Kühner	MW282494, MW282577, MW282651, MW282688, MW282689	X	X	
Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma bryorum* Romagn.	MW282338, MW282376, MW282389, MW282404, MW282431, MW282433, MW282436, MW282440, MW282463, MW282466, MW282479, MW282498, MW282502, MW282514, MW282536, MW282563, MW282588, MW282593, MW282602, MW282624, MW282626,	X	X	

Table S5.1 (cont.)

Basidiomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma bryorum* Romagn.	MW282650, MW282659, MW282660, MW282674, MW282684, MW282687, MW282700, MW282701, MW282711	X	X	
Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma griseorugulosum* Noordel. & Fern. Sas.	MW282481		X	
Agaricomycetes	Agaricales	Entolomataceae	*Entoloma*	*Entoloma* sp. 1	MW282430, MW282600	X		
Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria amethystina* Cooke	MW282555, MW282612, MW282615, MW282739	X		X
Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria laccata* (Scop.: Fr.) Cooke	MW282360, MW282448, MW282482, MW282507, MW282567, MW282592, MW282662, MW282691, MW282695, MW282728, MW282729	X	X	
Agaricomycetes	Agaricales	Hydnangiaceae	*Laccaria*	*Laccaria pumila* Fayod	MW282556			X
Agaricomycetes	Agaricales	Hymenogastraceae	*Hymenogaster*	*Hymenogaster hessei* Soehner	MW282476, MW282493		X	
Agaricomycetes	Agaricales	Hymenogastraceae	*Hymenogaster*	*Hymenogaster pruinatus* R. Hesse	MW282561		X	
Agaricomycetes	Agaricales	Tricholomataceae	*Tricholoma*	*Tricholoma sulphureum* (Bull.: Fr.) P. Kumm.	MW282664		X	
Agaricomycetes	Boletales	Boletaceae	*Cyanoboletus*	*Cyanoboletus pulverulentus* (Opat.) Gelardi, Vizzini & Simonini	MW282397, MW282401, MW282441, MW282492, MW282702	X	X	
Agaricomycetes	Boletales	Boletaceae	*Leccinum*	*Leccinum carpini* (R. Schulz) M.M. Moser ex D.A. Reid	MW282345			X
Agaricomycetes	Boletales	Paxillaceae	*Melanogaster*	*Melanogaster macrosporus* Velen.	MW282597, MW282670			X
Agaricomycetes	Boletales	Paxillaceae	*Melanogaster*	*Melanogaster* sp.1	MW282336, MW282437, MW282442, MW282473			X
Agaricomycetes	Boletales	Boletaceae	*Octaviania*	*Octaviania asterosperma* Vittad.	MW282462, MW282686			X
Agaricomycetes	Boletales	Boletaceae	*Phylloporus*	*Phylloporus pelletieri* (Lév.) Quél.	MW282347	X		
Agaricomycetes	Boletales	Boletaceae	*Xerocomellus*	*Xerocomellus porosporus* (Imler ex Watling) Šutara	MW282454		X	
Agaricomycetes	Boletales	Boletaceae	*Xerocomellus*	*Xerocomellus pruinatus* (Fr. & Hök) Šutara	MW282331, MW282414, MW282439, MW282483, MW282501, MW282568	X		X
Agaricomycetes	Boletales	Sclerodermataceae	*Scleroderma*	*Scleroderma citrinum* Pers.	MW282447, MW282525, MW282552	X		
Agaricomycetes	Cantharellales	Cantharellaceae	*Craterellus*	*Craterellus cornucopioides* (L.: Fr.) Pers.	MW282369, MW282410, MW282426, MW282521, MW282575	X		X

Table S5.1 (cont.)

Basidiomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Agaricomycetes	Cantharellales	Cantharellaceae	*Craterellus*	*Craterellus* sp. 1	MW282673			X
Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina cristata* (Holmsk.: Fr.) J. Schröt.	MW282372, MW282595, MW282714, MW282735	X		X
Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*	*Clavulina* sp. 1	MW282692, MW282671			X
Agaricomycetes	Cantharellales	Hydnaceae	*Hydnum*	*Hydnum ovoideisporum* Olariaga, Grebenc, Salcedo & M.P. Martín	MW282598, MW282654			X
Agaricomycetes	Cantharellales	Hydnaceae	*Hydnum*	*Hydnum repandum* L.: Fr.	MW282578			X
Agaricomycetes	Cantharellales	Hydnaceae	*Hydnum*	*Hydnum rufescens* Pers.: Fr.	MW282468, MW282491, MW282587			X
Agaricomycetes	Cantharellales	Hydnaceae	*Sistotrema*	*Sistotrema* sp. 1	MW282379, MW282523, MW282576, MW282581, MW282608, MW282652, MW282665, MW282667, MW282706, MW282722, MW282731			X
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius blennius* (Fr.: Fr.) Fr.	MW282384, MW282461, MW282590, MW282718			X
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius fuliginosus* (Fr.: Fr.) Fr.	MW282349, MW282419, MW282649, MW282655, MW282736, MW282738	X	X	X
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius pallidus* Pers.: Fr.	MW282648		X	
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius subdulcis* (Pers.: Fr.) Gray	MW282363, MW282370, MW282373, MW282399, MW282420, MW282422, MW282425, MW282457, MW282470, MW282474, MW282505, MW282506, MW282519, MW282530, MW282544, MW282572, MW282632, MW282683, MW282717	X	X	X
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius tabidus* Fr.	MW282333, MW282732	X		
Agaricomycetes	Russulales	Russulaceae	*Lactarius*	*Lactarius volemus* (Fr.: Fr.) Fr.	MW282453, MW282548			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula acrifolia* Romagn.	MW282486			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula amoenicolor* Romagn.	MW282666		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula chloroides* (Krombh.) Bres.	MW282346			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula delica* Fr.	MW282680	X		
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula faustiana* Sarnari	MW282394		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula fellea* (Fr.: Fr.) Fr.	MW282408, MW282475, MW282489, MW282565, MW282682	X		X

Table S5.1 (cont.)

Basidiomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula foetens* Pers.: Fr.	MW282417, MW282586, MW282645		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula ionochlora* Romagn.	MW282653,MW282668		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula melzeri* Zvára	MW282402, MW282522, MW282526, MW282630, MW282644, MW282681, MW282716	X	X	X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula nigricans* Fr.: Fr.	MW282339, MW282366, MW282449, MW282477, MW282503, MW282529, MW282560, MW282585, MW282616	X	X	X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula ochroleuca* Fr.	MW282591, MW282599	X		
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula pectinatoides* Peck	MW282594		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula praetervisa* Sarnari	MW282516	X		
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula puellula* Ebbesen, F.H. Møller & Jul. Schäff.	MW282446, MW282469, MW282513			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula risigallina* (Batsch) Sacc.	MW282367			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula rosea* Pers.	MW282344, MW282510, MW282627			X
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula solaris* Ferd. & Winge	MW282335, MW282609, MW282610		X	
Agaricomycetes	Russulales	Russulaceae	*Russula*	*Russula* sp. 1	MW282340, MW282356	X		
Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina helvelloides* (Schwein.: Fr.) Burt	MW282396		X	
Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina incrustans* (Pers.: Fr.) Tul. & C. Tul.	MW282368, MW282388	X	X	
Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina* sp. 1	MW282403, MW282495, MW282508, MW282518, MW282569, MW282582, MW282628	X		X
Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	*Sebacina* sp. 2	MW282705	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Pseudotomentella*	*Pseudotomentella* sp. 1	MW282504, MW282547	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*	*Thelephora* sp. 1	MW282694			X
Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*	*Thelephora* sp. 2	MW282385		X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella atroarenicolor* Nikol.	MW282678		X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella badia* (Link) Stalpers	MW282362, MW282549	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella bryophila* (Pers.) M.J. Larsen	MW282677, MW282690	X	X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella coerulea* Höhn. & Litsch.	MW282438, MW282451, MW282675	X		X

Table S5.1 (cont.)

Basidiomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella ferruginia* (Pers.: Fr.) Pat.	MW282500	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella lapida* (Pers.) Stalpers	MW282458, MW282484, MW282511, MW282676			X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella lateritia* Pat.	MW282539	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella lilacinogrisea* Wakef.	MW282393			X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 1	MW282342, MW282348, MW282359, MW282411, MW282412, MW282429, MW282435, MW282444, MW282452, MW282517, MW282540, MW282562, MW282571, MW282635, MW282672	X	X	X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 2	MW282490	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 3	MW282465			X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 4	MW282617. MW282551, MW282434			X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 5	MW282636		X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 7	MW282456		X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 8	MW282566	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella* sp. 9	MW282607, MW282710		X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella stuposa* (Link) Stalpers	MW282355, MW282407, MW282423, MW282485, MW282712	X	X	
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella sublilacina* (Ellis & Holw.) Wakef.	MW282382	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella subtestacea* Bourdot & Galzin	MW282478	X		
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella terrestris* (Berk. & Broome) M.J. Larsen	MW282512, MW282696, MW282725	X		X
Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella*	*Tomentella viridula* (Bourdot & Galzin) Svrček	MW282405, MW282413, MW282416, MW282708		X	
Eurotiomycetes	Eurotiales	Elaphomycetaceae	*Elaphomyces*	*Elaphomyces leucosporus* Vittad.	MW282637	X		
Eurotiomycetes	Eurotiales	Elaphomycetaceae	*Elaphomyces*	*Elaphomyces muricatus* Fr.: Fr.	MW282341, MW282534, MW282669	X		
Eurotiomycetes	Eurotiales	Elaphomycetaceae	*Elaphomyces*	*Elaphomyces* sp. 1	MW282395			X

Table S5.1 (cont.)

Ascomycota								
Class	**Order**	**Family**	**Genus**	**Species**	**GenBank accession no.**	**M1**	**M2**	**Out**
Leotiomycetes	Helotiales	Hyaloscyphaceae	*Meliniomyces*	*Meliniomyces bicolor* Hambl. & Sigler	MW282352, MW282579, MW282631		X	X
Dothideomycetes	Mytilinidiales	Gloniaceae	*Cenococcum*	*Cenococcum geophilum* Fr.: Fr.	MW282332, MW282343, MW282357, MW282358, MW282365, MW282378, MW282386, MW282387, MW282398, MW282406, MW282415, MW282432, MW282445, MW282460, MW282471, MW282520, MW282524, MW282527, MW282546, MW282570, MW282573, MW282580, MW282620, MW282633, MW282642, MW282699	X	X	X
Pezizomycetes	Pezizales	Discinaceae	*Hydnotrya*	*Hydnotrya tulasnei* (Berk.) Berk. & Broome	MW282361, MW282424, MW282509, MW282533, MW282603, MW282605, MW282622, MW282634, MW282723	X		X
Pezizomycetes	Pezizales	Helvellaceae	*Helvella*	*Helvella elastica* Bull.: Fr.	MW282724		X	
Pezizomycetes	Pezizales	Helvellaceae	*Helvella*	Helvella sp. 1	MW282693		X	
Pezizomycetes	Pezizales	Pezizaceae	*Hydnobolites*	*Hydnobolites* sp. 1	MW282428, MW282707		X	
Pezizomycetes	Pezizales	Pezizaceae	*Luteoamylascus*	*Luteoamylascus* sp. 1	MW282371, MW282375, MW282392, MW282487, MW282496	X	X	X
Pezizomycetes	Pezizales	Pezizaceae	*Pachyphlodes*	*Pachyphlodes conglomerata* (Berk. & Broome) Doweld	MW282351, MW282383, MW282497, MW282542, MW282611, MW282618, MW282623, MW282638, MW282656, MW282713, MW282719, MW282720, MW282721	X	X	X
Pezizomycetes	Pezizales	Pezizaceae	*Pachyphlodes*	*Pachyphlodes melanoxantha* (Tul. & C. Tul. ex Berk.) Doweld	MW282629, MW282726, MW282727, MW282734		X	
Pezizomycetes	Pezizales	Pezizaceae	*Pachyphlodes*	*Pachyphlodes nemoralis* Hobart, Bóna & A. Paz	MW282381, MW282450, MW282528, MW282553, MW282625, MW282640, MW282641, MW282657, MW282715	X		X
Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza michelii* (Boud.) Dennis	MW282679		X	
Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza* sp. 1	MW282354, MW282550, MW282709			X
Pezizomycetes	Pezizales	Pezizaceae	*Peziza*	*Peziza depressa* Pers.	MW282488, MW282558, MW282596, MW282621	X	X	
Pezizomycetes	Pezizales	Pezizaceae	*Plicariella*	*Plicariella scabrosa* (Cooke) Spooner	MW282531		X	
Pezizomycetes	Pezizales	Pyronemataceae	*Genea*	*Genea arenaria* Harkn.	MW282418		X	

Table S5.1 (cont.)

Pezizomycetes	Pezizales	Pyronemataceae	*Genea*	*Genea hispidula* Berk. ex Tul. & C. Tul.	MW282730, MW282733	X		
Pezizomycetes	Pezizales	Pyronemataceae	*Humaria*	*Humaria hemisphaerica* (F.H. Wigg.: Fr.) Fuckel	MW282499, MW282532, MW282583, MW282619, MW282643, MW282658, MW282697	X		
Pezizomycetes	Pezizales	Tuberaceae	*Tuber*	*Tuber borchii* Vittad.	MW282464	X		
Pezizomycetes	Pezizales	Tuberaceae	*Tuber*	*Tuber scruposum* R. Hesse	MW282535	X		

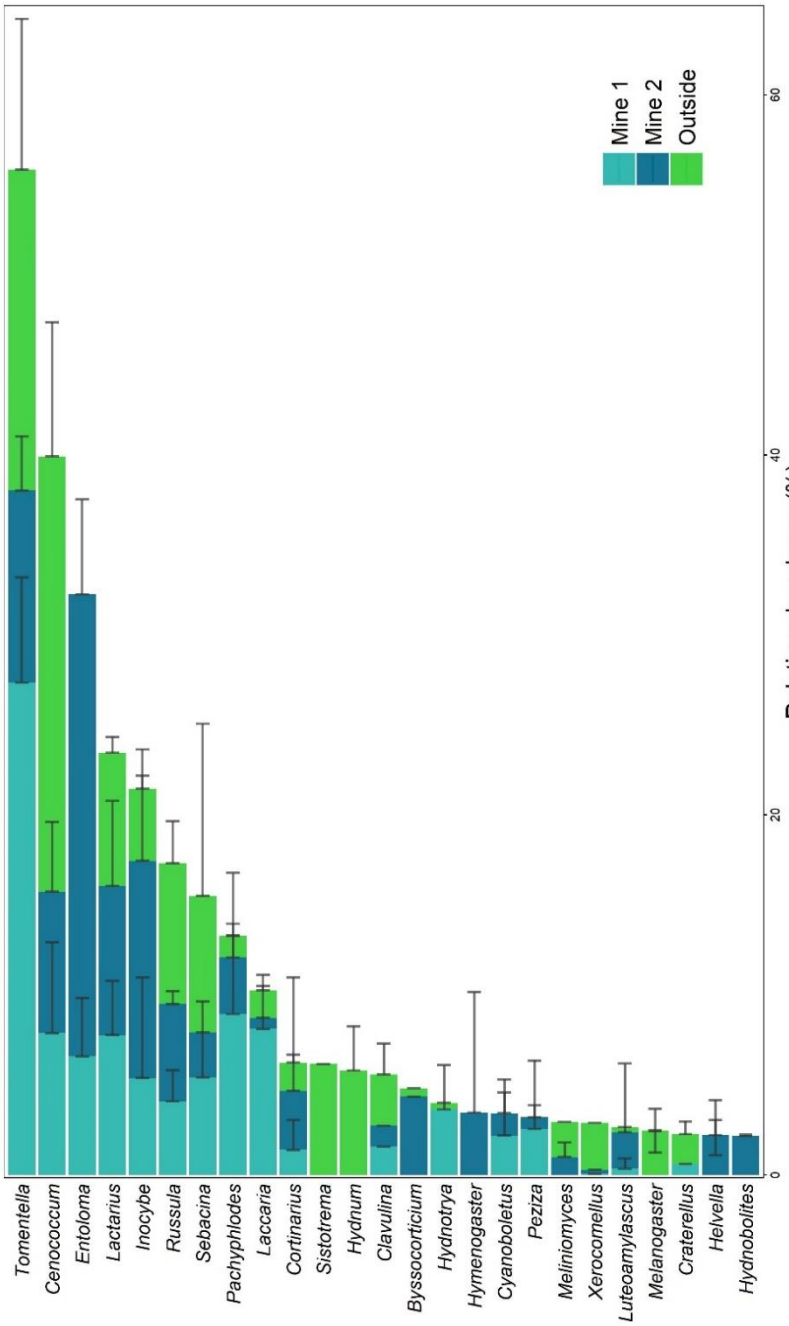


Figure S5.1. Relative abundances of ectomycorrhizal fungal genus, with >2% total relative abundance, from European beech roots present with and outside mines. Bars indicate means and standard error.

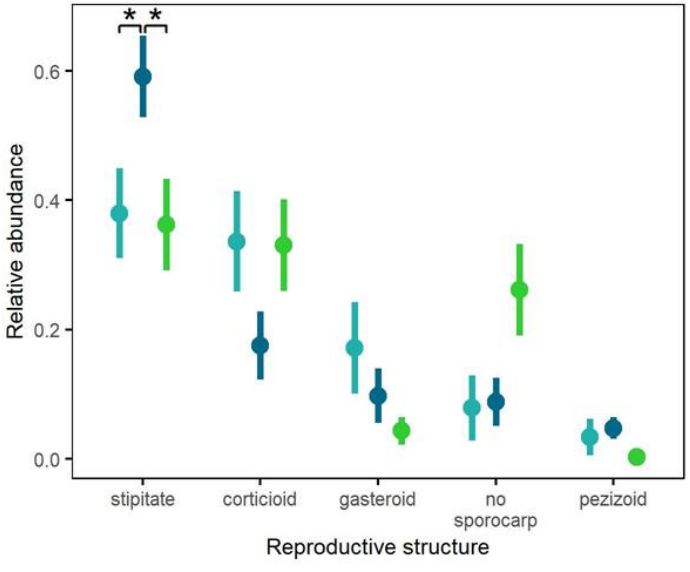
Table S5.2. Indicator ectomychorrizal fungal species from European beech roots for mine 1, mine 2 and outside both mines. A = positive predictive power, B = sensitivity, *Stat* = strength of the association. Significant *p*-values are shown in bold.

Location	EcM fungal species	A	B	*Stat*	*p*-value
Mine 1	*Laccaria laccata*	0.98	0.44	0.66	**0.02**
Mine 2	*Entoloma bryorum*	0.91	0.88	0.89	**<0.001**
Outside	*Cenococcum geophilum*	0.74	1.00	0.86	**0.01**

Table S5.3. Comparisons of the (a) generalized linear mixed models and the (b, c) linear mixed models fitted to explain variation of ectomycorrhizal fungi species richness, species diversity and taxonomic diversity, respectively, between mine 1, mine 2 and outside both mines. Df: degrees of freedom. AIC: Akaike Information Criterion. ΔAIC: AIC increase compared to the best model. The final model is shown in bold.

Model	(a) Species richness (Poisson)			(b) Species diversity			(c) Taxonomic diversity		
	AICc	ΔAIC	Df	AICc	ΔAIC	Df	AICc	ΔAIC	Df
y = 1 + (1 | tree)	**205.97**	**0**		**32.74**	**0**		**341.07**	**0**	
y = season + (1 | tree)	208.25	2.28	1	34.80	2.05	1	341.93	0.86	1
y = location + (1 | tree)	209.35	3.38	1	36.91	4.17	1	343.99	2.92	1
y = location + season + (1 | tree)	211.86	5.89	1	39.27	6.52	1	345.10	4.03	1
y = location * season + (1 | tree)	216.69	10.72	2	43.68	10.94	2	349.59	8.52	2

a)



Stipitate

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.14	0.07	1.29	**0.05**
Residuals	20	1.05	0.05		

Corticioid

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.19	0.10	2.63	0.10
Residuals	17	0.61	0.04		

Gasteroid

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.03	0.02	0.65	0.54
Residuals	17	0.46	0.03		

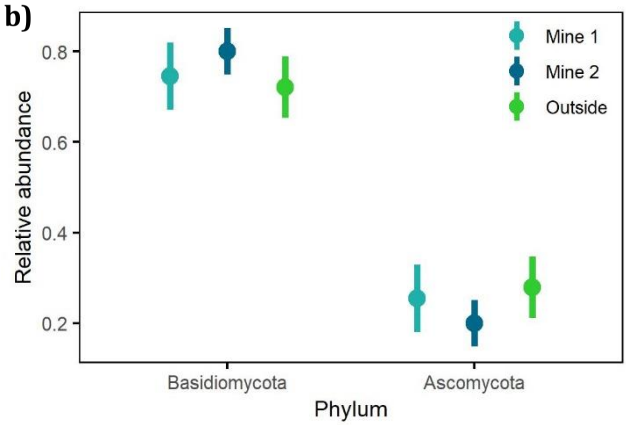
No sporocarp

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.07	0.04	1.65	0.23
Residuals	14	0.30	0.02		

Pezizoid

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.01	0.01	1.79	0.28
Residuals	4	0.01	0.003		

Figure S5.2 Mean relative abundance (%) (± standard error) of ectomycorrhizal fungi when grouped by (a) phylum, (b) exploration type, and (c) reproductive structure with and outside mines, and ANOVA results to test significant differences. Dots (.) indicate marginally significant differences ($p < 0.1$) obtained from Tukey post-hoc pairwise comparisons of the ANOVA results. SE: standard error. Significant *p*-values are shown in bold.



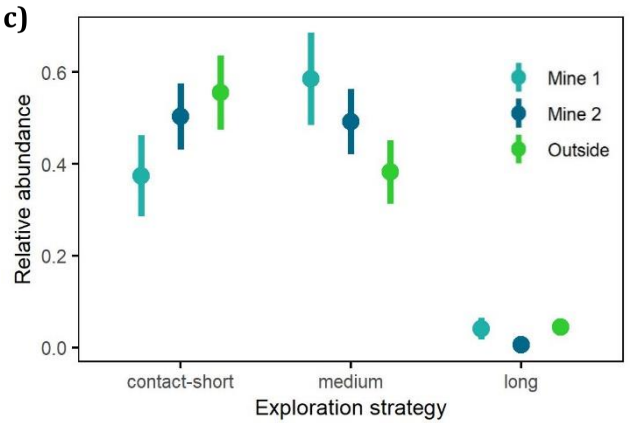


Figure S5.2 (cont.)

Basidiomycota

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.02	0.01	0.36	0.70
Residuals	20	0.68	0.03		

Ascomycota

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.02	0.01	0.36	0.70
Residuals	20	0.68	0.03		

Contact-short

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.14	0.07	1.29	0.30
Residuals	20	1.05	0.05		

Medium

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.15	0.07	1.28	0.30
Residuals	20	1.16	0.06		

Long

Source	Df	Sum Sq	Mean Sq	*F*-statistic	*p*-value
Location	2	0.04	0.02	5.04	0.06
Residuals	5	0.02	0.004		

Appendix S6. Changes in soil biogeochemistry

Table S6.1. ANOVA results to test the effect of location in each soil variable. Significant *p*-values are shown in bold. Sum Sq: sum of squares. Mean Sq: mean of squares.

pH					
Source	**Df**	**Sum Sq**	**Mean Sq**	***F*-statistic**	***p*-value**
Location	2	5.22	2.61	11.38	**<0.001**
Residuals	20	4.59	0.23		
C					
Source	**Df**	**Sum Sq**	**Mean Sq**	***F*-statistic**	***p*-value**
Location	2	38.48	19.24	3.27	0.06
Residuals	20	117.86	5.89		
ln (P)					
Source	**Df**	**Sum Sq**	**Mean Sq**	***F*-statistic**	***p*-value**
Location	2	5.17	2.59	6.36	**<0.001**
Residuals	20	8.13	0.40		
ln (NH_4)					
Source	**Df**	**Sum Sq**	**Mean Sq**	***F*-statistic**	***p*-value**
Location	2	13.41	6.71	11.40	**<0.001**
Residuals	20	11.77	0.59		
ln (NO_3)					
Source	**Df**	**Sum Sq**	**Mean Sq**	***F*-statistic**	***p*-value**
Location	2	3.65	1.82	1.05	0.37
Residuals	20	34.86	1.74		

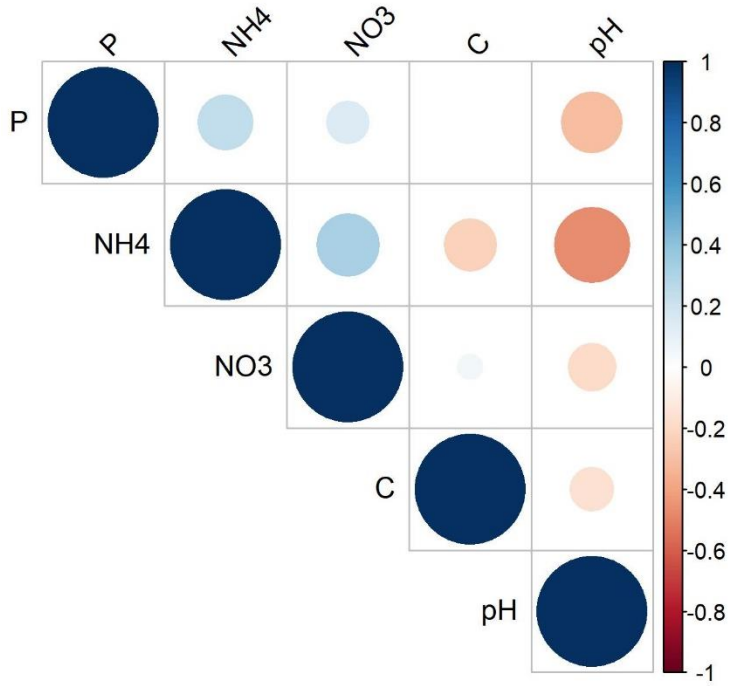


Figure S6.1. Pearson correlation among five soil variables measured. The size of the circle indicates the magnitude of the correlation and the colour defines the direction of the relationship. There is a significant negative correlation (ρ = -0.46, p = 0.03) between pH and NH_4.

Table S6.2. Results of the linear models to test the effect of soil variables on ectomycorrhizal fungi species richness, species diversity and taxonomic diversity. SE: standard error. Significant *p*-values are shown in bold.

Species richness				
Predictor variable	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	9.09	7.77	1.17	0.26
C	0.22	0.31	0.73	0.48
pH	-0.05	1.28	-0.04	0.97
P	0.02	0.10	0.25	0.84
NH_4	-0.01	0.03	-0.53	0.61
NO_3	0.01	0.02	0.52	0.61
Species diversity				
Predictor variable	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	1.75	0.77	2.26	**0.04**
C	0.001	0.03	0.03	0.98
pH	0.009	0.13	1.07	0.94
P	0.004	0.01	0.48	0.64
NH_4	-0.0002	0.002	-0.08	0.94
NO_3	-0.0001	0.001	-0.09	0.93
Taxonomic diversity				
Predictor variable	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	49.98	13.01	3.76	**0.002**
C	0.40	0.53	0.76	0.46
pH	2.73	2.19	1.24	0.23
P	0.08	0.17	0.51	0.62
NH_4	-0.02	0.05	-0.51	0.62
NO_3	-0.02	0.03	-0.78	0.45

Table S6.3. Results of the canonical correspondence analysis (CCA) to test the effect of the soil variables measured on ectomycorrhizal fungal interactions.

	Axis 1	**Axis 2**	**Axis 3**
Axis summary statistics and % of variance explained by CCA ordination			
Eigenvalue	0.71	0.56	0.53
% of variance explained	26.00	20.55	19.57
Cumulative % explained	26.00	46.55	66.12
Axis correlation with soil variables			
pH	0.99	-0.12	-0.08
C	-0.30	0.04	0.81
P	-0.04	0.40	0.42
NH_4	-0.39	0.73	-0.41
NO_3	-0.14	-0.35	-0.212

Supplementary information of Chapter 5

Appendix S7. Recovery of Collembola community structure

Table S7.1. Number of specimens of the Collembola species sampled at each location.

Collembola species	Family	Mine 1	Mine 2	Outside
Isotomiella minor Schaeffer, 1896	Isotomidae	3293	67	112
Folsomia manolachei Bagnall, 1939	Isotomidae	1150	80	273
Folsomia setosa Gisin, 1953	Isotomidae	954	4	217
Protaphorura armata Tullberg, 1869	Onychiuridae	28	4	691
Isotomiella madeirensis Gama , 1959	Isotomidae	189	12	104
Paratullbergia callipygos Borner, 1903	Tullbergiidae	143	12	89
Tomocerus minor Uchida, 1954	Tomoceridae	148	3	24
Isotomurus pseudopalustris Carapelli et al., 2001	Isotomidae	13	68	47
Pseudisotoma monochaeta	Isotomidae	14	6	26
Deuteraphorura silvaria Gisin, 1952	Onychiuridae	10	15	18
Pseudachorutes palmiensis Börner, 1903	Neanuridae	3	12	6
Pseudosinella sp. 1	Entomobryidae	6	2	11
Kalaphorura tuberculata Moniez, 1890	Onychiuridae	22	1	
Sminthurinus elegans Fitch, 1863	Katiannidae	7	7	34
Dicyrtomina ornata H.Nicolet, 1842	Dicyrtomidae	8	5	
Xenyllodes armatus W.M.Axelson, 1903	Odontellidae	3	6	
Ceratophysella tergilobata P.Cassagnau, 1954	Hypogastruridae		6	6
Heteromurus major Moniez, 1889	Bourletiellidae	5		14
Folsomia trisetata Jordana & Ardanaz, 1981	Isotomidae	176		
Parisotoma notabilis Schaeffer, 1896	Isotomidae	143		
Folsomia sp.	Isotomidae	32		
Protachorutes pyrenaeus Cassagnau, 1955	Neanuridae	21		
Entomobrya nicoleti Lubbock, 1868	Entomobryidae	20		
Isotomiella sp.	Isotomidae	10		
Symphypleona (order)		10		

Table S7.1. (Cont.)

Collembola species	Family	Mine 1	Mine 2	Outside
Kalaphorura sp.	Onychiuridae	6		
Lepidocyrtus lignorum O.Fabricius, 1775	Entomobryidae	5		
Bourletiella sp.	Bourletiellidae	4		
Friesea truncata P.Cassagnau, 1958	Neanuridae	3		
Cryptopygus sp. 2	Isotomidae		95	
Pseudosinella sp. 2	Entomobryidae		20	
Unknown			10	
Isotomurus fucicolus H.Schött, 1893	Isotomidae		6	
Cryptopygus sp. 1	Isotomidae		2	
Sminthurinus aureus Lubbock, 1862	Katiannidae		2	
Stenognathellus denisi P.Cassagnau, 1953	Katiannidae		2	
Folsomia ocellata Jordana, 1980	Isotomidae			34
Sminthurinus sp. 2	Katiannidae			7
Rusekella sp.	Neanuridae			5
Neanura muscorum R.Templeton, 1836	Neanuridae			1

Table S7.2. Results of the fitted models for the effect of location (mine 1, mine 2 and outside) on the species richness and diversity of Collembola communities. SE: standard error. Statistically significant *p*-values are shown in bold.

Collembola species richness (Poisson)				
Location	**Estimate**	**SE**	***z*-statistic**	***p*-value**
Intercept	1.90	0.16	12.00	**<0.001**
Mine 1	-0.02	0.20	-0.08	0.94
Mine 2	-0.37	0.23	-1.60	0.11
Collembola species diversity				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	1.54	0.20	7.79	**<0.001**
Mine 1	-0.25	0.26	-0.97	0.34
Mine 2	-0.16	0.25	-0.61	0.55

Table S7.3. Results of canonical correspondence analysis (CCA) to test the effect of the soil variables measured on Collembola communities inside and outside the mines.

	Axis 1	**Axis 2**	**Axis 3**
Axis summary statistics and % of variance explained by CCA ordination			
Eigenvalue	0.50	0.35	0.31
% of variance explained	34.56	24.43	21.09
Cumulative % explained	34.56	58.99	80.09
Axis correlation with soil variables			
pH	-0.84	-0.66	-0.03
C	0.18	-0.26	0.08
P	0.50	-0.13	0.30
NH_4	0.44	0.18	0.69
NO_3	-0.36	0.56	0.71

Appendix S8. Differences in Collembola diets

Table S8.1. Most abundant fungal OTUs identified in collembolan guts and their functional groups. Includes the number of networks by location where they were present. Values in brackets indicate the total number of networks constructed for each location.

Taxonomy	Functional group	Mine 1 (9)	Mine 2 (8)	Outside (6)
Helotiales 1	saprotroph	8	4	5
Helotiales 2	saprotroph	9	5	4
Helotiales 3	saprotroph	8	4	5
Cordyceps confragosa (Mains) G.H. Sung et al.	parasitic	7	3	5
Mycosphaerellaceae 1	parasitic	7	3	6
Helotiales 4	saprotroph	9	2	4
Helotiales 5	saprotroph	6	1	5
Helotiales 6	saprotroph	5	2	2
Helotiales 7	saprotroph	6	3	3
Helotiales 8	saprotroph	5	1	3
Helotiales 9	saprotroph	5	4	1
Helotiales 10	saprotroph	1	1	4
Gliocladium sp.	parasitic	6	2	3
Resinicium friabile Hjortstam & Melo	saprotroph	6	1	2
Lewia ethzedia E.G. Simmons	parasitic	4	1	3
Helotiales 11	saprotroph	3	2	2
Lecanorales 1	lichen	4	2	1
Helotiales 12	saprotroph	2	2	3
Mortierella humilis Linnem. ex W. Gams	saprotroph	2	1	4
Lecanicillium fungicola (Preuss) Zare & W. Gams	parasitic	3	3	1
Trichoderma citrinoviride Bissett	parasitic	4	2	2
Rhodosporidium aff. lusitaniae Á. Fonseca & J.P. Samp.	saprotroph	2	1	4
Botrytis tulipae (Lib.) Lind	parasitic	3	1	2

Table S8.1. (Cont.)

Taxonomy	Functional group	Mine 1 (9)	Mine 2 (8)	Outside (6)
Mortierella exigua Linnem.	saprotroph	2	2	2
Trichoderma viride Pers.	parasitic	2	2	2
Cantharellales 1	mycorrhiza	1	1	3
Helotiales 13	saprotroph	2	1	2
Hydnum repandum L.	mycorrhiza	1	2	1
Mortierella cystojenkinii W. Gams & Veenb.-Rijks	saprotroph	2	1	1
Mortierella macrocystopsis W. Gams & Veenb.-Rijks	saprotroph	1	2	1
Helotiales 14	saprotroph	1	1	2
Dictyochaeta sp.	saprotroph	5	1	
Cantharellales 2	mycorrhiza	3	1	
Helotiales 15	saprotroph	2	2	
Helotiales 16	saprotroph	2		1
Pochonia bulbillosa (W. Gams & Malla) Zare & W. Gams	parasitic	5		3
Helotiales 17	saprotroph	2		1
Hirsutella sp.	parasitic	4		1
Porosphaerella cordanophora E. Müll. & Samuels	saprotroph	1		1
Neonectria sp.	parasitic	2		2
Helotiales 18	saprotroph	2		1
Amyloporia xantha (Fr.) Bondartsev & Singer	parasitic	3		2
Cantharellales 3	mycorrhiza	2		1
Ramalina crispatula Despr. ex Nyl.	lichen	2		1
Ascochyta pinodes L.K. Jones	parasitic		4	1
Helotiales 19	saprotroph	4		
Helotiales 20	saprotroph	3		
Helotiales 21	saprotroph	3		

Table S8.2. Results of the fitted model for the effect of location (mine 1, mine 2 and outside) on the species richness of Collembola-associated fungi. SE: standard error. Statistically significant *p*-values are shown in bold.

Fungal species richness (Poisson)				
Location	**Estimate**	**SE**	***z*-statistic**	***p*-value**
Intercept	3.78	0.06	61.31	**<0.001**
Mine 1	0.34	0.07	4.53	**<0.001**
Mine 2	-0.89	0.10	-8.59	**<0.001**

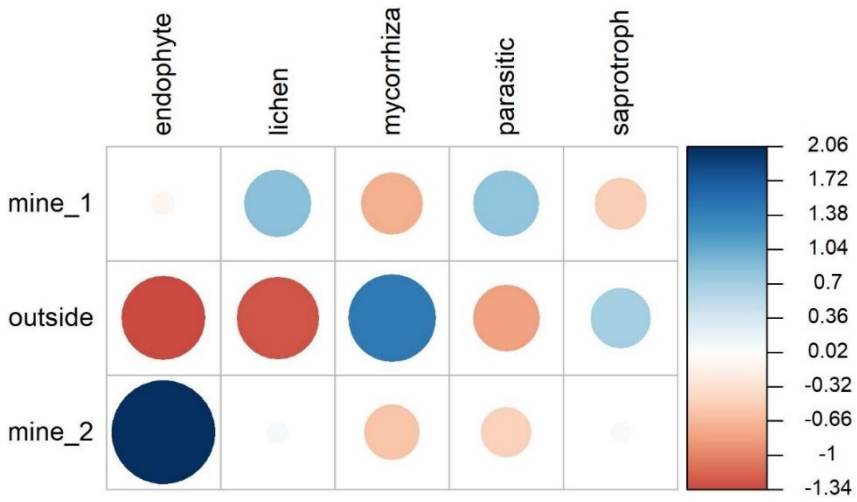


Figure S8.1. Visualization of Pearson residuals from chi-squared test to detect associations between the fungal functional groups and the three locations. The size of the circle indicates the magnitude of the association and the colour defines the direction of the relationship.

Appendix S9. Recovery of Collembola-fungi network architecture

Table S9.1. Results of the fitted models for the effect of location (mine 1, mine 2 and outside) on the network metrics calculated, except for robustness metrics. SE: standard error. Df: degrees of freedom. Statistically significant *p*-values are shown in bold and marginally significant p-values are shown in italics.

Number of species (Poisson)				
Location	**Estimate**	**SE**	***z*-statistic**	***p*-value**
Intercept	3.92	0.06	68.27	**<0.001**
Mine 1	0.29	0.07	4.26	**<0.001**
Mine 2	-0.80	0.09	-8.55	**<0.001**
Number of links				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	54.50	12.01	4.53	**<0.001**
Mine 1	21.28	15.50	1.37	0.19
Mine 2	-33.37	15.88	-2.10	**0.05**
Connectance				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	-1.69	0.17	-9.69	**<0.001**
Mine 1	0.11	0.23	0.48	0.64
Mine 2	0.42	0.23	1.83	*0.08*
Modularity				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.63	0.05	11.51	**<0.001**
Mine 1	-0.08	0.07	-1.12	0.27
Mine 2	-0.06	0.07	-0.86	0.40
Nestedness (Kruskal-Wallis test)				
Predictor variable		**Df**	**X^2**	***p*-value**
Location		2	3.54	0.17

Table S9.2. Number of networks from each location with nestedness (NODF) and modularity (Q) values significantly lower (z <-1.96) or higher (z >-1.96) than the null distribution of values obtained for 100 random networks and the corresponding mean z-scores (mean z) for each location. Values in brackets indicate the total number of networks constructed for each location.

NODF	***z* <-1.96**	***z* >1.96**	**mean *z***
Mine 1	3 (9)	1 (9)	1.44
Mine 2	6 (8)	1 (8)	-3.97
Outside	2 (6)	1 (6)	-1.32
Q	***z* <-1.96**	***z* >1.96**	**mean *z***
Mine 1	2 (9)	7 (9)	0.853
Mine 2	1 (8)	3 (8)	1.00
Outside	0 (6)	5 (6)	3.01

Table S9.3. Results of the fitted models for the effect of location (mine 1, mine 2 and outside) on the robustness metrics calculated, except for robustness metrics. SE: standard error. Statistically significant *p*-values are shown in bold.

Robustness (random)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.82	0.03	31.45	**<0.001**
Mine 1	0.05	0.03	1.33	0.20
Mine 2	-0.05	0.03	-1.50	0.15
Robustness (least abundant)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.90	0.03	35.43	**<0.001**
Mine 1	0.04	0.03	1.33	0.20
Mine 2	-0.07	0.03	-2.13	**0.05**
Robustness (best connected)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.90	0.03	35.43	**<0.001**
Mine 1	0.01	0.07	0.1	0.92
Mine 2	-0.14	0.07	-2.05	**0.05**
Functional robustness (random)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.54	0.01	56.54	**<0.001**
Mine 1	0.01	0.01	0.48	0.64
Mine 2	-0.01	0.01	-0.37	0.72
Functional robustness (least abundant)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.73	0.03	22.23	**<0.001**
Mine 1	0.03	0.04	0.64	0.53
Mine 2	-0.05	0.04	-1.10	0.29
Functional robustness (best connected)				
Location	**Estimate**	**SE**	***t*-statistic**	***p*-value**
Intercept	0.33	0.03	9.83	**<0.001**
Mine 1	-0.001	0.04	-0.02	0.99
Mine 2	0.01	0.04	0.13	0.90

"Lo que yo percibo en la Naturaleza es una estructura magnífica que solo podemos comprender muy imperfectamente, y eso debe llenar a cualquier persona de un sentimiento de humildad."

(Albert Einstein)