PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
Facultad de Ciencias Biológicas
Programa de Doctorado en Ciencias Biológicas
Mención Ecología

TESIS DOCTORAL

Causes and consequences of Nitrogen limited chronosequences: evidence from the Andean Dry Puna

Por

FERNANDO DANIEL ALFARO AYLLON

Marzo 2014

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
Facultad de Ciencias Biológicas
Programa de Doctorado en Ciencias Biológicas
Mención Ecología

Causes and consequences of Nitrogen limited chronosequences: evidence from the Andean Dry Puna

Tesis presentada a la Pontificia Universidad Católica de Chile como parte de los requisitos para optar al grado de Doctor en Ciencias Biológicas con mención en Ecología.

Por

FERNANDO DANIEL ALFARO AYLLON

Director de tesis:	Pablo A. Marquet
Co-Director de tesis	Aurora Gaxiola
Comisión de tesis:	Juan Armesto
	Ramiro Bustamante

Marzo 2014

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
Facultad de Ciencias Biológicas
Programa de Doctorado en Ciencias Biológicas
Mención Ecología

La defensa final de la tesis Doctoral titulada:

Causes and consequences of Nitrogen limited chronosequences: evidence from the Andean Dry Puna

Presentada por el candidato a Doctor en Ciencias Biológicas
Mención Ecología

FERNANDO DANIEL ALFARO AYLLON

Ha sido aprobada por el Tribunal Examinador, constituido por los profesores abajo firmantes, calificándose el trabajo realizado, el manuscrito sometido y la defensa oral con nota _____

Dr. Pablo A. Marquet
Director de Tesis
Facultad de Ciencias Biológicas-PUC

Dr. Juan A. Correa M.
Decano
Facultad de Ciencias Biológicas-PUC

Dr. Aurora Gaxiola
Co-director de Tesis
Facultad de Ciencias Biológicas-PUC

Dr. Juan Armesto
Miembro Comisión de Tesis
Facultad de Ciencias Biológicas-PUC

Dr. Claudio Latorre
Jefe de la Mención
Facultad de Ciencias Biológicas-PUC

Dr. Ramiro Bustamante
Miembro Externo Comisión de Tesis
Universidad de Chile

Santiago de Chile, 14 de Marzo de 2014

INDICE

RESUMEN…… viii

INTRODUCCIÓN GENERAL…..……... 1

Marco teórico…….……..…... 1

El problema y la pregunta...……..…..... 7

Referencias……...……..……. 9

CAPÍTULO I…..……...……...…….. 17

Abstract……..……... 18

Introduction..…..…… 19

Methods……...…..…. 22

Results……..……..…… 28

Discussion…...……..…. 33

Acknowledgements……... 42

Literature cited…….. 43

CAPÍTULO II…….. 69

Abstract……..…...……..……...70

Introduction……..…….…… 71

Methods…….…… 74

Results……..……..…… 76

Discussion……. 81

Acknowledgements……...… 86

Literature cited…….……. 87

CAPÍTULO III……. 108

Summary…….……... 109

Introduction…… 111

Material and methods…… 115

Results…… 119

Discussion…… 124

Concluding remarks…… 130

Acknowledgements…… 132

References...…… 133

CONCLUSIONES GENERALES……..159

A Danny, Lalo, Ely, Vlady y Thiago

AGRADECIMIENTOS

Mediante las siguientes líneas intentare plasman mi profundo agradecimiento a las personas e instituciones que contribuyeron para que yo pueda llegar a este punto.

Al profesor Pablo Marquet por brindarme la oportunidad de trabajar en su laboratorio, lugar en donde pude interactuar con científicos destacados de todo el mundo; además de aprender y comprender que la ciencia se hace con mucha pasión.

A Aurora Gaxiola por su enorme paciencia para guiarme durante la tesis y por su claridad para comprender los distintos procesos en el mundo de las cronosecuencias.

A los miembros de la comisión evaluadora, Angélica González, Juan Armesto y Ramiro Bustamante por sus valiosos aportes y sugerencias.

A las instituciones que me permitieron realizar mis estudios en la Pontificia Universidad Católica de Chile y mi estadía Chile. Al Fondo Mundial para la Conservación (WWF) Becas Russell E. Train Education for Nature. A Prince Bernhard Scholarship Fund for Nature Conservation (PBS-WWF). A CONICYT, la beca para estudios de doctorado a estudiantes latinoamericanos y la beca de apoyo a tesis de doctorado. A la Vicerrectoría de Investigación (VRI-PUC) y la Dirección de Investigación y Postgrado (DIP-PUC).

Un agradecimiento especial al Instituto de Ecología y Biodiversidad (IEB) por su colaboración durante el desarrollo de la tesis y mi estadía durante el último año en Chile.

Un agradecimiento especial al Centro de Biodiversidad y Genética (CBG) y a la Unidad de Limnología y Recursos Acuáticos (ULRA) de la Universidad Mayor de San Simón que colaboraron durante los viajes a terreno.

Al Laboratorio de Ecología Celular, donde se realizaron los análisis meta-genómicos, fundamentalmente a Marlene Manzano por su enorme voluntad y pasión para enseñar, por su forma de ver el mundo y por sobre todo por su amistad.

A Angélica, Feñita, Sebas y Andy por su consejo y apoyo durante el desarrollo de la tesis.

A todos los miembros del Lab Marquet.

A mis amigos de la legión extranjera en Chile, Fernanda y Felipe; Ana y Sergio y a Caro y Guille por todos los buenos momentos compartidos.

A mis amigos de todo la vida Coral, Ale, Renzo, Cesar, Saúl, Moi y José.

A Sinzi, señora Gabriela y David por el apoyo constante.

A las hermanas Morales por formar la mejor familia.

Al gran salar de Tunupa y las comunidades de Colcaya, Llica, Castilluma, Tahua, Salinas de Garci Mendoza, Coipasa y los esforzados amigos de la isla Incahuasi. A Iván y Filtson por su colaboración durante los extensos viajes de campo.

A ustedes Paola Villarroel, Huber Villca, Alejandro Coca y Mauricio Torrico, en verdad fue un gran privilegio haber dirigido sus trabajos de investigación. Sin duda, esta tesis no se hubiera logrado sin su incondicional apoyo.

A Ely y Vlady por hacerme sentir orgulloso siempre.

A Danny y Lalo por ser lo más importante en mi vida.

A Thiago por ser la alegría de estos últimos años.

A ti Dana por todo!!

RESUMEN

Se ha establecido que en ambientes mésicos el cambio en el contenido de nutrientes durante el desarrollo a largo plazo de los suelos determina los cambios en procesos ecosistémicos tales como la productividad primaria y el reciclaje de nutrientes, así como en la composición y diversidad de plantas y microorganismos del suelo. Por lo tanto, suelos jóvenes limitados por Nitrógeno (N) están dominados por plantas de crecimiento rápido y rápida descomposición, y en estados tardíos de desarrollo el Fósforo (P) se vuelve limitante y las especies cambian a estrés-tolerantes, de crecimiento lento y bajas tasas de descomposición. Sin embargo, estudios recientes en ambientes áridos muestran que la limitación por N domina los procesos ecosistémicos, por lo que la trayectoria de desarrollo de los suelos podría ser significativamente distinta a lo observado en regiones mésicas. En este trabajo se evaluaron las causas y consecuencias del desarrollo de los suelos en ambiente áridos limitados por N y su efecto sobre las comunidades de plantas y microorganismos del suelo a lo largo de tres cronosecuencias de c. 20,000 años con suelos derivados a partir de diferente material parental en la Puna seca de los Andes centrales de Bolivia. Los resultados confirman que el N es limitante a lo largo del gradiente de edad, mientras que el P se mantuvo sin cambios, este patrón se mantuvo independientemente del origen geológico de los suelos de las cronosecuencias. El incremento en la razón N:P durante el desarrollo de los suelos determinó los incrementos en la riqueza de especies, la descomposición y la translocación de nutrientes en plantas que se observaron en las tres cronosecuencias. Las comunidades de bacterias y hongos del suelo mostraron patrones contrastantes; la abundancia de bacterias incrementó con la edad de los suelos, pero la abundancia de hongos declinó a lo largo del desarrollo de los suelos, lo que sugiere un cambio en la dominancia microbiana en relación con el cambio en la disponibilidad de nutrientes a lo largo del gradiente de edad de los suelos.

En conjunto estos resultados indican que a diferencia de los ambientes mésicos, en ambientes limitados permanentemente por el agua, la limitación por N retarda el desarrollo de los suelos, y de los componentes bióticos sobre y debajo del suelo, manteniendo así a los ecosistemas en un estado de “juventud” permanente.

INTRODUCCIÓN GENERAL

Marco teórico

Los procesos biogeoquímicos que gobiernan el desarrollo de los suelos y la composición, estructura y funcionamiento de los ecosistemas terrestres a lo largo del tiempo, han estado en el centro de la investigación en ecología desde sus inicios (Gleason, 1917; Crocker & Major, 1955; Odum, 1969). El estrecho vínculo entre los procesos que ocurren simultáneamente debajo del suelo "belowground" y sobre la superficie "aboveground" determina que los cambios en procesos ecosistémicos tales como la producción primaria, descomposición y el reciclaje de nutrientes, reflejen cambios en atributos del suelo tales como el contenido de nutrientes, pH, la actividad y biomasa microbiana (Wardle et al, 2004b; Bardgett et al, 2005; Gaxiola et al, 2010). Sin embargo, estos cambios a nivel ecosistémico ocurren a escalas de tiempo que van más allá del tiempo de vida de cualquier investigador, ya que en su mayoría exceden los cientos y miles de años (Wardle, 2002). Por esta razón, las cronosecuencias edáficas se han constituido como el modelo paradigmático en la ecología de ecosistemas ya que permiten estudiar los cambios biogeoquímicos del suelo y los patrones ecológicos (e.g. sucesión de la vegetación), así como los procesos ecosistémicos sobre escalas temporales que alcanzan los millones de años (Wardle et al, 2012). Las cronosecuencias edáficas *sensu stricto,* se definen como el "conjunto de sitios (o etapas) formados a partir del mismo material parental, pero que difieren en la edad a la cual fueron colonizados por organismos y por lo tanto en la etapa de desarrollo ecosistémico" (Walker et al, 2010).

A partir de los patrones observados en cronosecuencias edáficas con distintos orígenes tales como retiros de glaciales (Walker & Syers, 1976; Chapin et al, 1994; Richardson et al, 2004), actividad volcánica (Crews et al, 1995; Vitousek et al, 2004),

deposición de arena (Walker et al, 1981; Laliberté et al, 2012) y levantamientos por tectónica de placas (Coomes et al, 2005; Gaxiola et al, 2010), particularmente en regiones templadas y sub-tropicales (ver Peltzer et al, 2010) se ha enfatizado en el rol crítico de nutrientes tales como el Nitrógeno (N) y el Fósforo (P) (Wardle et al, 2004a) en los procesos ecosistémicos a lo largo del tiempo (Fig. 1). El modelo de Walker & Syers (1976) para ecosistemas templados establece que las distintas propiedades químicas y físicas de los nutrientes determinan los cambios en las abundancias relativas, por ejemplo de N y P, así como las formas químicas en las que se encuentran presentes durante el desarrollo de los suelos. Asimismo, el modelo explica que estos cambios afectan los mecanismos a través de los cuales el N y P fluyen a través de los distintos componentes de los ecosistemas (e.g. suelos, plantas). En particular, las diferentes formas en las que se encuentran el N y P de manera natural determinan su balance y disponibilidad, tanto para plantas como para los microorganismos durante el desarrollo de los suelos (Peltzer et al, 2010).

El mayor aporte de N en los suelos proviene desde la atmósfera, a partir de la actividad de bacterias simbiontes y bacterias de vida libre (Sprent & Sprent, 1990; Vitousek et al, 2002b). Por esta razón suelos jóvenes o recientemente perturbados presentaran bajos contenidos de N, que irán en incremento con la actividad biológica a lo largo del desarrollo de los suelos (Vitousek et al, 2002). En cambio, dado que el P no presenta una fase gaseosa, y que la mayor parte de este se encuentra en forma de Fosfato de Calcio (Apatita), el material parental representa la principal fuente de P para el ecosistema (Walker & Syers, 1976). De esta manera, suelos jóvenes presentan la máxima cantidad de P que tendrá ese ecosistema durante su desarrollo. En otras palabras, cada ecosistema “nace” con el total de P en el sustrato parental y éste se va liberando por acción de factores abióticos (lixiviación) y actividad biótica. A lo largo de estos procesos el P va cambiando de formas moleculares

pasando por fosfatos, que son de fácil adquisición para las plantas, a fósforo orgánico, que no es útil para las plantas (Vitousek et al, 2010). El P se pierde fácilmente durante el desarrollo de los suelos y la mayor parte queda inmovilizado con iones de Al+ y Fe+ en suelos húmedos (Walker & Syers, 1976; Vitousek et al, 2010). Por esta razón, suelos muy antiguos sin perturbaciones recientes presentan bajos contenidos de P disponible para las plantas (Fig. 1).

De acuerdo con los modelos descritos para sistemas templados y tropicales, la transición desde la limitación por N (etapas tempranas) hasta la limitación por P (etapas tardías) a lo largo del desarrollo de los suelos está relacionada con importantes cambios en el desarrollo de los ecosistemas, a través de la modificación de procesos ecosistémicos tales como el reciclaje de nutrientes y la descomposición (Crews et al, 1995; Wardle et al, 2004a); lo cual a su vez también tiene efectos en la estructura, composición y diversidad funcional de las comunidades bióticas durante el desarrollo de los suelos (Coomes et al, 2005; Wardle et al, 2008; Jandid et al, 2013). Por ejemplo, suelos recientemente perturbados donde los procesos ecosistémicos comienzan desde cero, estarán dominados por especies vegetales de crecimiento rápido, que producen materia orgánica con altas concentraciones de nutrientes, que promueven rápidas tasas de descomposición y liberación de nutrientes (Wardle et al, 2004b). De esta manera, muchos procesos ecosistémicos incrementan hasta alcanzar una fase máxima de productividad en donde N y P co-limitan tales procesos. Posteriormente, los ecosistemas entran en una fase en donde comienza la limitación de P, que a menudo está acompañada por un recambio de especies de plantas hacia comunidades dominadas por especies menos productivas y de crecimiento lento que producen materia orgánica con bajas concentraciones de nutrientes y lenta descomposición(Fig. 2) (Richardson et al, 2004; Wardle et al, 2004a). Un aspecto

fundamental que subyace estos patrones es que la máxima productividad alcanzada durante el desarrollo de los ecosistemas no puede ser mantenida indefinidamente a lo largo del tiempo en ausencia de grandes perturbaciones que permitan “reiniciar” el proceso (Wardle et al, 2004a; Peltzer et al, 2010).

Más allá de la importancia de los cambios a nivel comunitario (recambio de especies) durante el desarrollo de los suelos, también están los cambios a nivel de rasgos foliares tales como el contenido de nutrientes en la hojas (Richardson et al, 2004), la reabsorción de nutrientes desde las hojas, tiempo de vida de las hojas, y cambios en la densidad especifica de las hojas (Escudero et al, 1992; Killingbeck, 1996). Los cambios en los rasgos foliares a lo largo del desarrollo de los suelos controlan los procesos de descomposición y reciclaje de nutrientes (Richardson et al, 2005). Por ejemplo, en estados avanzados de desarrollo ecosistémico las especies conservan nutrientes más eficientemente, por lo tanto producen hojarasca de baja calidad (i.e. alto Carbono:Nutrientes y Lignina:Nutrientes) que tiende a descomponerse lentamente permitiendo la acumulación de grandes cantidades de material recalcitrante (Richardson et al, 2005) que modifican el pH del suelo y promueven en anegamiento y la inmovilización de P en Aluminio (Gaxiola et al, 2010). Estos cambios en la calidad de la materia orgánica que llega al suelo, también afectan la actividad y los patrones de productividad y composición de las comunidades microbianas del suelo, tales como bacterias y hongos (Bardgett et al, 2005; Eskelinen et al, 2009; Espershütz et al, 2011). Es así, que en los últimos años evidencia proveniente de estudios en cronosecuencias edáficas que abarcan escalas temporales cortas (0-200 años) enfatizan la estrecha relación entre los patrones de producción de biomasa microbiana y la calidad de la materia orgánica generada por las comunidades de plantas (Espershütz et al, 2011; Schulz et al, 2013). En particular, se ha sugerido que las comunidades microbianas a

menudo están asociadas con ensambles específicos de plantas, y que cambios en la composición de plantas durante el desarrollo de los suelos están acompañados por cambios en la estructura y composición de grupos de bacterias y hongos del suelo (Porazinska et al, 2003; Jandid et al, 2013; Williams et al, 2013).

A partir de los trabajos de Wardle et al, (2004; 2008) donde se comparan los patrones de múltiples cronosecuencias de zonas boreales y subtropicales, se ha sugerido un rol crucial del contenido de P en los suelos sobre variadas propiedades de los ecosistemas (e.g. productividad primaria) durante estadios tardíos del desarrollo de los suelos. Sin embargo, las tasas en la que estos procesos ocurren dependerán de factores tales como el clima (Selmants & Hart, 2010), material parental de los suelos (Chadwick & Chorover, 2001; Vitousek et al, 2010) y el tipo de vegetación entre otros (Kitayama, 2005). Por ejemplo, evidencia proveniente de cronosecuencias en zonas áridas sugiere que los patrones de contenido de N y P durante el desarrollo de los suelos podrían no seguir las dinámicas propuestas por Walker & Syers (1976), e incluso se proponen que la limitación por N se extiende por tanto tiempo que la limitación por P no se presenta (Lajtha & Schlesinger, 1988; Carreira & Lajtha, 1997). Lo anterior ocurriría particularmente en ambientes donde la actividad microbiana (bacterias fijadoras de N) está restringida a pequeños y esporádicos eventos de precipitación, por lo que la entrada "input" de N al sistema es muy restringida (Hooper & Johnson, 1999; Yahdjian & Sala, 2010; Delgado-Baquerizo et al, 2013). Este fenómeno podría ser aún más extremo en ambientes áridos de altura (FAO, 2011), como los que se encuentran en la Puna Árida del centro de Sudamérica. En estos sitios la baja entrada de N al sistema está acoplada con elevadas pérdidas de N por vías de desnitrificación asociadas a la rápida volatilización del N desde suelo. Por lo tanto, ambientes áridos podrían permanecer en un perpetuo estado de limitación por N y sin

continuar la trayectoria de desarrollo biogeoquímico y ecosistémico sugeridas por Walker & Syers (1976) para sistemas mésicos.

De igual manera, se ha sugerido que las diferencias observadas en el funcionamiento ecosistémico y en los patrones de diversidad a lo largo del desarrollo de los suelos estarían determinadas por efectos del material parental que dan origen a los suelos (Huggett, 1998; Lichter, 1998; Rasmussenet al, 2007; Vitousek et al, 2010). Sin embargo, los estudios de cronosecuencias edáficas publicados en los últimos 15 años no han reportado cronosecuencias generadas en diferente material parental pero que hayan estado sujetas a las mismas condiciones climáticas. Por lo tanto, el efecto del material parental en los ciclos biogeoquímicos de N y P a lo largo del desarrollo del suelo no ha sido evaluado. Esto es de gran relevancia, porque cronosecuencias con suelos derivados a partir de distintos materiales podrían seguir tendencias de desarrollo muy distintas. Por ejemplo, suelos de origen volcánico tienden a formar alófanos, ya que tienen alta concentración de compuestos ricos en Aluminio (Borie & Zunino, 1983). En estos suelos el P es rápidamente adsorbido en formas químicas de muy difícil accesibilidad para plantas y microorganismos (Deubel & Merbach, 2005). Por lo tanto, suelos derivados a partir de material volcánico podrían formar diferentes formas de P que aquellos suelos sedimentarios (Escudey et al, 2001; Lilienfein et al, 2003) y la limitación por P puede ocurrir a una escala de temporal menor que en suelos de otro origen, aun cuando las condiciones climáticas fueran las mismas. Estas diferencias inclusive podrían presentarse entre tipos de materiales volcánicos (Yavitt, 2000; Escudey et al, 2001). Asimismo, el pH de los suelos afecta no solamente la disponibilidad de nutrientes en el suelo sino también la actividad de microorganismos tales como las bacterias (Kemmitt et al, 2006; Rousk et al, 2009), además que el pH varia significativamente entre suelos de origen volcánico. Por lo tanto, existen varios factores a

través de los cuales los patrones de desarrollo de los suelos podrían diferir significativamente entre cronosecuencias volcánicas y sedimentarias.

Por lo tanto, los efectos no considerados del tipo de material parental a partir del cual se derivan los suelos, podrían determinar que el desarrollo de los suelos con diferente origen geológico aún bajo las mismas condiciones ambientales, presenten tasas de desarrollo distintas y fuera del patrón predicho por los modelos clásicos como los de Walker & Syers (1976). Finalmente, es importante desatacar que cerca del medio centenar de cronosecuencias reportadas en los últimos 15 años, solamente tres se encuentran en sistemas semiáridos, cada una con patrones de desarrollo de los suelos muy contrastantes, por lo tanto, la aplicabilidad del concepto de desarrollo de suelos propuesta por Walker & Syers (1976) es incierta.

El problema y la pregunta

Las generalizaciones entorno a la transición desde una limitación de N a una de P provienen a partir de evidencia recopilada en ambientes húmedos, donde el balance de P en el suelo tiende a ser negativo debido a que las pérdidas por lixiviación e intemperización exceden por mucho las pérdidas de N durante estados tardíos del desarrollo de los suelos (Fig. 1). En cambio en aquellos ambientes inherentemente empobrecidos en N como los ambientes áridos y semiáridos, esta transición (limitación de N a P) podría ser mucho más lenta o inclusive, no presentarse nunca (Menge et al, 2012). De esta manera los patrones de comunidades de plantas y microorganismos, además de procesos ecosistémicos tales como la productividad primaria, descomposición y reciclaje de nutrientes durante el desarrollo a largo plazo de los suelos podrían ser ampliamente distintos entre ambientes permanentemente limitados por agua y ambientes húmedos (Fig. 2)

Por lo tanto, este trabajo de investigación apunta a responder la siguiente pregunta general: ¿Son los patrones del desarrollo de los suelos y sus efectos sobre el desarrollo de los ecosistemas en donde las tasas del ciclo de N se encuentren limitadas, distintos a aquellos observados en ambientes húmedos donde las tasas del ciclo de P son muy altas?; y si así fuera, cuáles son los principales atributos ecológicos y ecosistémicos que caracterizan están diferencias?

Referencias

Bardgett, R. D., Bowman, W. D., Kaufmann.R., andS. K. Schmidt. 2005. A temporal approach to linking aboveground and belowground ecology. Trends in Ecology & Evolution.20: 634–41.

Borie, F., and H. Zunino.1983. Organic matter-phosphorus associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biology and Biochemistry. 15: 599-603.

Carreira, J. A., and K. Lajtha. 1997. Factors affecting phosphate sorption along a Mediterranean, dolomitic soil and vegetation chronosequence. European Journal of Soil Science, 48: 139-149.

Chadwick, O. A., and J. Chorover. 2001. The chemistry of pedogenic thresholds. Geoderma. 100: 321-353.

Chapin, F. S., Walker, L. R., Fastie, C. L., and L. C. Sharman. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecological Monographs. 64: 149-175.

Crews, T. E., et al.1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. Ecology. 76: 1407-1424.

Coomes, D. A., et al. 2005. The hare, the tortoise and the crocodile: the ecology of angiosperm dominance, conifer persistence and fern filtering. Journal of Ecology. 93: 918-935.

Crocker, R. L., and J. Major. 1955. Soil development in relation to vegetation and surface age at Glacier Bay, Alaska. Journal of Ecology. 43:427-448.

Delgado-Baquerizo, M., et al. 2013. Aridity Modulates N Availability in Arid and Semiarid Mediterranean Grasslands.PLoS One. 8: 59807-59814.

Deubel.A., W. Merbach. 2005. Influence of Microorganisms on Phosphorus Bioavailability in Soils. In: F. Buscot and A. Varma (eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag, Berlin Heidelberg, Germany.

Escudero, A., J. M. Del Arco, I. C. Sanz, and J. Ayala. 1992. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. Oecologia. 90: 80-87.

Escudey, M., et al. 2001. Chemical forms of phosphorus of volcanic ash-derived soils in Chile. Communications in Soil Science and Plant Analysis. 32: 601-616.

Eskelinen, A., S. Stark, and M. Männistö. 2009. Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. Oecologia 161: 113–23.

Esperschütz, J., A. et al. 2011. Microbial food web dynamics along a soil chronosequence of a glacier forefield. Biogeosciences Discussions. 8: 1275-1308.

FAO, Mountain Partnership Secretariat, UNCCD, SDC, CDE. 2011. Highlands and Drylands – mountains, a source of resilience in arid regions. Published by FAO, UNCCD, Mountain Partnership, Swiss Agency for Development and Cooperation, and CDE, with the support of an international group of experts. Rome.

Gaxiola, A., McNeill, S. M., and D. A. Coomes. 2010. What drives retrogressive succession? Plant strategies to tolerate infertile and poorly drained soils.Functional Ecology. 24: 714-722.

Gleason, H. A. 1917. The structure and development of the plant association. Bulletin of the Torrey Botanical Club. 44: 463-481.

Hooper, D. U., and L. Johnson. 1999. Nitrogen limitation in dryland ecosystems: responses

to geographical and temporal variation in precipitation. Biogeochemistry. 46: 247-293.

Huggett, R. 1998. Soil chronosequences, soil development, and soil evolution: a critical review. Catena 32:155–172.

Kemmitt, S. J., Wright, D., Goulding, K. W., and D. L. Jones. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry. 38: 898-911.

Killingbeck, K. T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology. 77: 1716-1727.

Kitayama, K. 2005. Comment on "Ecosystem properties and forest decline in contrasting long-term chronosequences". Science. 308: 633-634

Lajtha, K., and W. H. Schlesinger. 1988. The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. Ecology. 69: 24-39.

Laliberté, E., et al. 2012. Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. Journal of Ecology 100:631–642.

Lilienfein, J., Qualls, R. G., Uselman, S. M., and S. D. Bridgham. 2003. Soil formation and organic matter accretion in a young andesitic chronosequence at Mt. Shasta, California. Geoderma. 116: 249-264.

Lichter, J. 1998. Rates of weathering and chemical depletion in soils across a chronosequence of Lake Michigan sand dunes. Geoderma, 85: 255-282.

Menge, D. N., Hedin, L. O., and S. W. Pacala. 2012. Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. PloS One. 7: 42045-42071.

Odum, E. P. 1969. The strategy of ecosystem development. Science 164: 262-270.

Peltzer, D. A., et al. 2010. Understanding ecosystem retrogression. Ecological Monographs. 80: 509-529.

Porazinska, D. L., Bardgett, R. D., Blaauw, M. B., Hunt, H. W., Parsons, A. N., Seastedt, T. R.,and D.H.Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecological Monographs. 73: 377-395.

Rasmussen, C., Matsuyama, N., Dahlgren, R. A., Southard, R. J., and N.Brauer. 2007. Soil genesis and mineral transformation across an environmental gradient on andesitic lahar.Soil Science Society of America Journal. 71: 225-237.

Richardson, S. J., et al. 2004. Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence:267–276.

Richardson, S. J., D. A. Peltzer, R. B.Allen, and M. S. McGlone. 2005. Resorption proficiency along a chronosequence: responses among communities and within species. Ecology. 86: 20-25.

Rousk, J., Brookes, P. C., and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology. 75: 1589-1596.

Schulz, S., Brankatschk, R., Dümig, A., Kögel-Knabner, I., Schloter, M., and J. Zeyer. 2013. The role of microorganism at different stages of ecosystem development for soil formation. Biogeosciences. 10: 3983-3996.

Selmants, P. C. and S. C. Hart. 2010. Phosphorus and soil development: does the Walker and Syers model apply to semiarid ecosystems? - Ecology 91: 474–84.

Sprent, J. I., and P. Sprent. 1990. Nitrogen Fixing Organisms. Chapman and Hall, London.

Vitousek, P. M., S. Hättenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and

nature. AMBIO: A Journal of the Human Environment. 31: 97-101.

Vitousek, P. M., et al. 2002b. Towards an ecological understanding of biological nitrogen fixation Biogeochemistry 58:1–45.

Vitousek, P. M. 2004. Nutrient cycling and limitation: Hawaiias a model system. Princeton University Press, Princeton,New Jersey, USA.

Vitousek, P. M., Porder, S., Houlton, B. Z., and O. A. Chadwick. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications. 20: 5-15.

Walker, T. W., and J. K. Syers. 1976. The fate of phosphorus during pedogenesis. Geoderma. 15: 1-19.

Walker, J., Thompson, C. H., Fergus, I. F., and B. R. Tunstall. 1981. Plant succession and soil development in coastal sand dunes of subtropical eastern Australia. In Forest Succession (pp. 107-131).Springer New York.

Walker, L. R., D. A. Wardle, R. D. Bardgett, and B. D. Clarkson. 2010. The use of chronosequences in studies of ecological succession and soil development Journal. of Ecology 98:725–736.

Wardle, D. A. 2002. Islands as model systems for understanding how species affect ecosystem properties. Journal of Biogeography. 29: 583-591.

Wardle, D.A., Hornberg, G., Zackrisson, O., Kalela-Brundin, M. and D. A. Coomes. 2003. Long-term effects of wildfire on ecosystem properties across an island area gradient.Science. 300: 972–975.

Wardle, D. A., Walker, L. R., and R. D. Bardgett. 2004a. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science. 305: 509-513.

Wardle, D. A., et al. 2004b. Ecological linkages between aboveground and belowground

biota. Science. 304: 1629-1633

Wardle, D. A. et al. 2008. The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. Oikos 117: 93–103.

Wardle, D. A., et al. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. Journal of ecology. 100: 16-30.

Williams, M. A., Jangid, K., Shanmugam, S. G.and W. B. Whitman. 2013. Bacterial communities in soil mimic patterns of vegetative succession and ecosystem climax but are resilient to change between seasons. Soil Biology and Biochemistry. 57: 749-757.

Yahdjian, L., and O. E. Sala. 2010. Size of precipitation pulses controls nitrogen transformation and losses in an arid Patagonian ecosystem. Ecosystems. 13: 575-585.

Yavitt, J. B. 2000. Nutrient dynamics of soil derived from different parent material on Barro Colorado Island, Panama1. Biotropica. 32: 198-207.

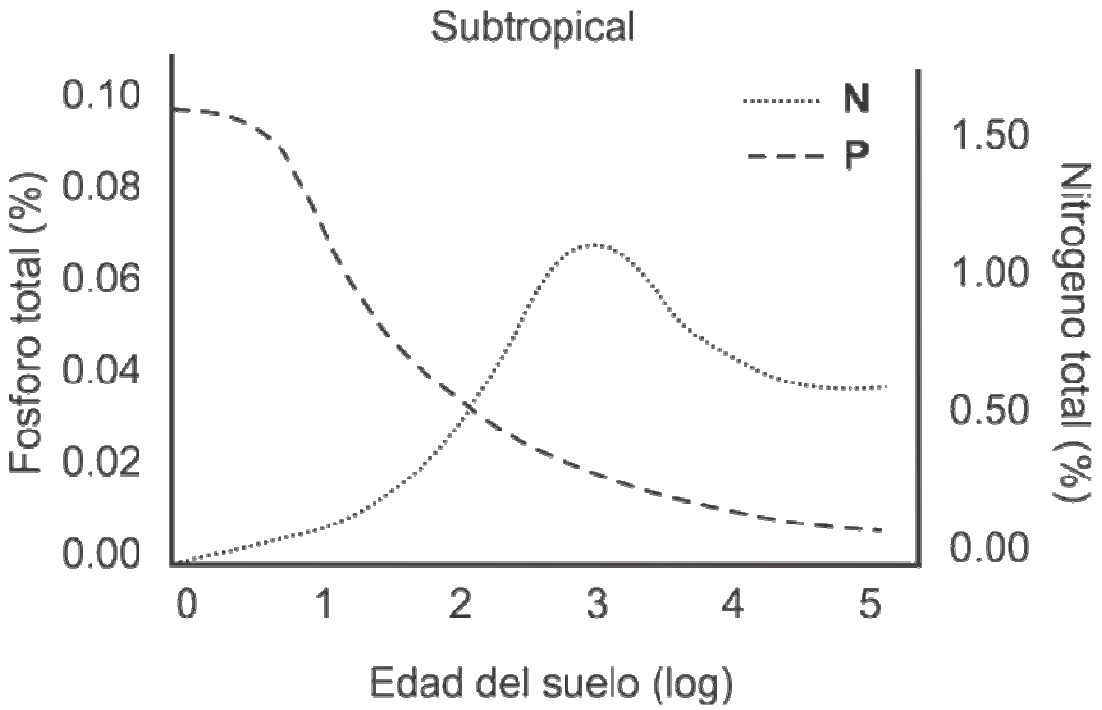


Figura 1. Patrones de N y P durante el desarrollo de los suelos a largo plazo. Las curvas fueron generadas a partir de la información presentada en Richardson et al, 2004 y que corresponden a una cronosecuencia formada por el retiro del glaciar en Franz Josef, en una región húmeda de la isla sur de Nueva Zelanda.

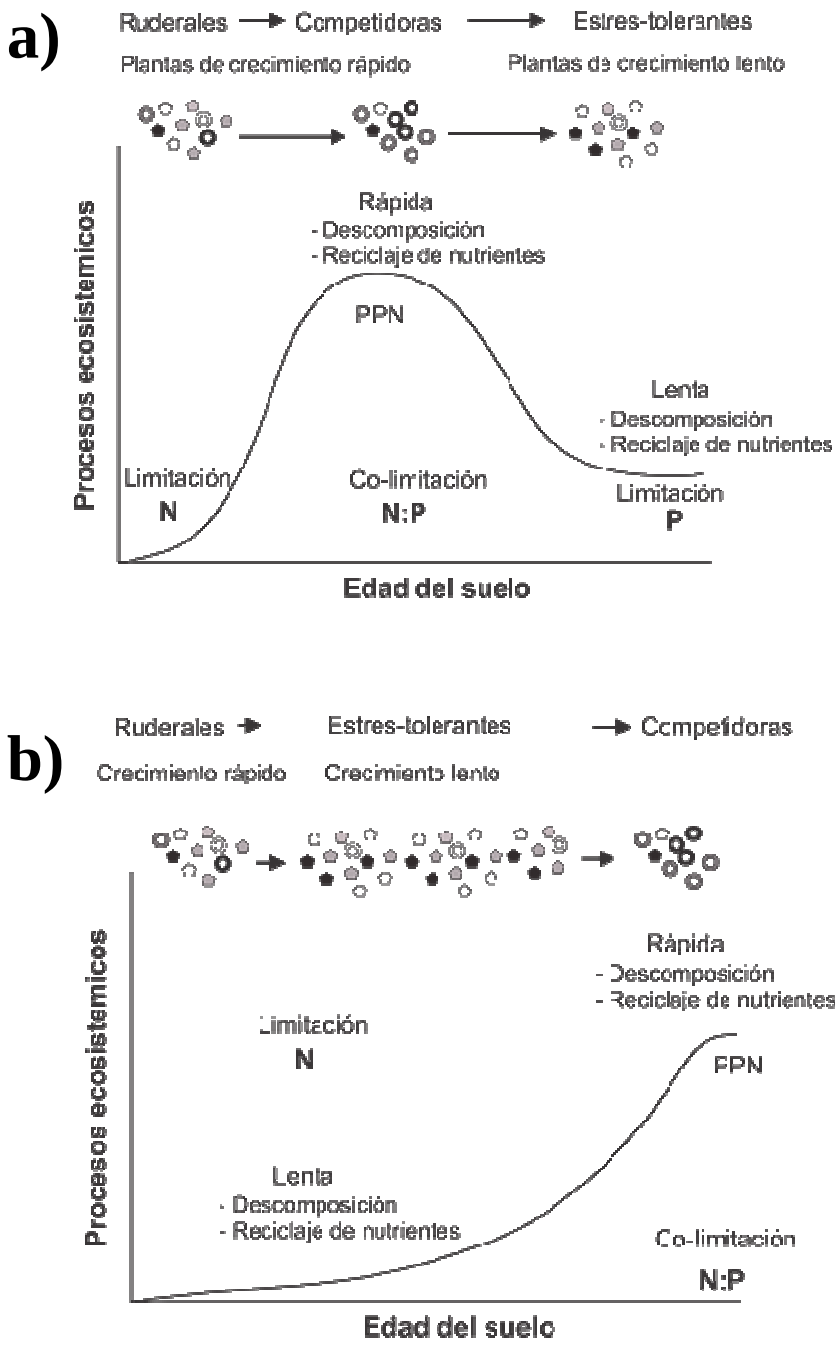


Figura 2. Resumen de los cambios esperados en los procesos ecosistémicos y la composición de comunidades de plantas a lo largo del desarrollo de los suelos en ambientes **a)** mésicos y **b)** áridos. La curva representa los cambios en la productividad primaria y las tasas de descomposición y reciclaje de nutrientes a lo largo del desarrollo de los suelos.

CAPÍTULO I

Hyper slow soil development after catastrophic disturbance: soil N-limitation is the source of eternal youth in long-term successions in the Central Dry Andes

ABSTRACT

It is widely accepted that in mesic environments that during soil development nutrient limitation on ecosystem process such as primary productivity, decomposition and nutrient cycling turn from Nitrogen (N) on youngest stages to Phosphorous (P) during the late stages of soil development. However, evidence from arid environments indicates disparate patterns of soil P during soil development and suggests that N limitation could overcome the effects of P limitation across long-term pedogenesis. Here, we evaluated the patterns of soil development in three primary successional c. 20,000-years chronosequences with soils derived from different parent material in the Andean Dry Puna, Bolivia. Further, we assessed the effect of changes in soil properties during soil development on plant biomass production, decomposition and nutrient resorption. Our results indicate that soil development was largely affected by strong N-limitation in all chronosequences, and its effects propagated across all stages of development. However, the rates of development were significantly different among chronosequences, with sedimentary chronosequence showing the lowest rate. Soil conditions improved with soil age, with increases in N:P and decline in soil pH and salinity during late stages of development, and this contributed to increase decomposition and nutrient content in leaf and litter of plants. Plan cover showed a unimodal pattern in all chronosequences with the second stage of development (~ 11,000 yr) showing the highest values. Notwithstanding the observed increases in soil N and plant leaf and litter N during soil development, these values were much lower than those recorded in chronosequences from mesic environments with similar time of development. Thereby, several lines of evidence strongly suggest that chronosequences in the Uyuni-SF have extreme slow soil development after ca. 20,000 years, and that soil N-limitation drive this process across chronosequences with soils derived from distinct parent material.

INTRODUCTION

After catastrophic disturbances major changes in ecosystem processes across soil development, such as primary productivity, Nitrogen (N) and Phosphorus (P) availability and decomposition rates, are largely dependent on variation in soil chemical and physical conditions (Wardle et al., 2004; Gaxiola et al., 2010; Walker et al., 2010). Based in the model proposed by Walker and Syers (1976) several studies have emphasized the critical role of soil P limitation on ecosystems processes during late stages of soil ecosystem development, particularly in mesic environments (Richardson et al., 2004; Wardle et al, 2004; Laliberté et al., 2012). Soil N limitation; in contrast, occurs during early stages of soil development (the build-up phase *sensu* Wardle et al, 2003). However, evidence from arid environments indicates disparate patterns of soil P during soil development (Lajtha and Schlesinger, 1988; Carreira and Lajtha, 1997; Carreira et al., 1997) suggesting that N-limitation could retard P-limitation across long-term soil development. Therefore, the discussion about how soil nutrients regulate ecosystem functioning during long-term soil development in arid and semiarid environments remains open. In particular, because large changes in primary productivity and other ecosystem processes are expected in these environments in the next 50 years in response to increased atmospheric N-deposition associated to elevated temperatures and declines in precipitation (Sanz et al., 2002; Fenn et al., 2003; Galloway et al., 2008).

In semiarid ecosystems, Nitrogen, second to water, is considered a key limiting resource for primary productivity as well as for decomposition and nutrient cycling (Noy-Meir, 1973; Hooper and Johnson, 1999; Yahdjian et al, 2011). In semiarid ecosystems low N-availability has been associated with extreme temperatures and dry conditions, which hampers the metabolic activity of Nitrogen-fixers, as well as microbial communities that

decompose and mineralize organic matter (Gallardo and Schlesinger, 1992; Austin, 2011). In high altitude dry ecosystems, such as Dryland Mountain Ecosystems (DMEs), the activity of N-fixing organisms is limited, as the energetic costs for the Nitrogenase enzyme activity are higher at low oxygen and low temperatures (Houlton et al., 2008). Moreover, in high altitude ecosystems, evaporation and low air pressure promote denitrification due to rapid N volatilization (Evans and Ehleringer, 1993; Baron et al., 1994). Therefore, ecosystem processes such as nutrient cycling and decomposition in DMEs may be limited by N-availability (Taylor et al., 1991; Taylor, 1998; Drewnik, 2006), in turn, determining that soil and ecosystem development occurs at very slow rates. An example of these, rarely studied DMEs, is the Andean Dry Puna (ADP), a large plateau over 3600 m.a.s.l. that represents the most extensive DME in South America (Sarmiento, 1975; Baied and Wheeler, 1993) and the fourth in the world (FAO, 2011). Soils in the ADP are poorly developed with very low content of organic matter and nutrients; in particular N is largely the scarcest nutrient in these soils (Navarro and Ferreira, 2004; Cardenas and Choque, 2008), with total-N values ranging from 0.02 (%) to 0.16 (%) (Cary and Angulo, 2006; Urcelay et al., 2011). In comparison, grasslands ecosystems range between 1.20 (%) and 3.18 (%) (Fornara and Tilman, 2008) and temperate rainforests between 0.01 (%) to 1.8 (%) (Richardson et al., 2004; Gallardo et al., 2012). Therefore, in contrast to soil development processes in mesic environments, in the ADP environments soil N-limitation could hinder soil development after catastrophic disturbances, retaining ecosystems in a permanent "youth" state; this idea has never been tested as yet.

Differences in soil biogeochemical transformations between semiarid and mesic ecosystems may be confounded by the effects of climate on soil weathering. However, growing evidence points out to parent material as an important driver of soil nutrient

dynamics and biogeochemical processes during soil development (Anderson, 1988; Huggett, 1998; Chadwick et al., 1999). In long-term soil development, as parent materials become exposed, weathering and microbial communities release minerals available for plant and microbial nutrition (Lichter, 1998; Espershütz et al., 2011). As soil development proceeds, the interaction between parent material and soil microbial activity is key for the bioavailability of substrate-derived nutrients such as Phosphorus (Chadwick and Chorover, 2001; Deubel and Merbach, 2005). Similarly nutrient fixation by soil microbial communities may also be intrinsically determined by parent material transformations during soil development (Anderson, 1988; Bannert et al., 2011). For example, diversity and abundance of microbial organisms involved in N-fixation changed following soil modifications across soil development in the Damma Glacier Forefield (Töwe et al., 2010). Similarly geochemically different soils, related to different parent material, determined important changes in community structure and functional diversity of rhizospheric bacterial communities in arable soils (Ulrich and Becker, 2005). Therefore, soil formation and ecosystem properties after disturbances strongly depend on geochemical properties of soils as well as on the origin of the parent material. To our knowledge; the influence of parent material on patterns of nutrient availability across long-term soil development has never been examined, and even less so in semiarid ecosystems. A logical extension of all the research done in long-term soil chronosequences is to link changes in N and P availability in soil chronosequences of different parent material and contrast any patterns with those found along mesic sequences, and more importantly keeping climate conditions constant.

Although N-limitation in young stages and P-limitation in old stages of soil chronosequences are commonly observed in mesic environments (Wardle et al., 2004; Laliberte et al., 2012), not all young soils are N-limited (Richardson et al., 2004) and not

only P-limitation reduces plant productivity in old soils (Gaxiola et al., 2010; Vitousek et al., 2010). Therefore, species identities as well as soil conditions play a key role in the "speed" at which soils develop and the stage these reach. Nutrient limitation, when is not experimentally proven via fertilization experiments (Vitousek and Farrington,1997) is often evaluated indirectly from leaf and litter nutrient contents, which are leaf-level parameters often used to estimate nutrient limitation (Escudero et al., 1992; Wright and Westoby, 2003).

Here, we took advantage of a natural experiment to explore how shifts in soil conditions, in particular N availability, along chronosequences of different parent material but subject to the same climatic conditions, affect plant individual (e.g. leaf and litter chemistry) and community (e.g. productivity) patterns, as well as ecosystem processes such as decomposition, along soil development in Andean Dry Puna ecosystems. Our study was designed to address these major questions: (1) Is soil N content the major limiting factor to ecosystem processes during soil development in ADP? (2) Are plant leaf and litter nutrient concentrations good indicators of soil nutrient availability across soil development? (3) Does nutrient resorption from senescent leaves increase with soil nutrient limitation during soil development? and (4) Are ecosystem processes, in terms of plant biomass production and litter decomposition (mass loss) across soil development explained by changes in soil properties.?

METHODS

Natural experiment: the chronosequence

In the Andean Dry Puna or Salt Puna (*sensu* Troll, 1968) located in the central Andes of South America, there is a chronosequence system that is a natural long-term experiment.

This chronosequence system is on an extended plateau at 3,600 m.a.s.l. in the southern boundary of the Andean Puna close to the Atacama Desert. The chronosequence system of this study is an archipelago of islands with parent material of different geological origin immersed in the Salar de Uyuni, or Uyuni salt flat (Uyuni-SF) whose basin encompasses 11,400 km^2 at 20°10 S and 67° 39 W (Figure 1). These islands not only differ in geological origin, but also in area and elevation (ABC, 2001; Andrade et al., 2006), although slope is relatively similar among islands (Table 1). Some islands are volcanic and were formed during Miocene to Pliocene periods from different parent material and chemical composition of rocks. Other islands were formed of sedimentary deposits (SGM, 2002; Tibaldi et al., 2009) (Table 1).

Uyuni-SF represents the latest of four salt-lakes that covered this region during the last 20,000 years (Risacher and Fritz 1991), which have been associated with dramatic oscillations in precipitation throughout the Pleistocene in the Andean Dry Puna (Placzek et al., 2009; 2011). One such oscillation occurred in the period between 18,000-8,000 years (BP) (Latorre et al., 2006) and is referred as the Central Andean Pluvial Event (CAPE) (Quade et al., 2008) and contributed to the formation of an extensive and deep paleolake in the endorheic Uyuni basin (Baker et al., 2001; Placzek et al., 2009). Throughout the development of CAPE two maximum lake expansion events have been identified (Placzek et al., 2011). The first and oldest event called Tauca cycle (18,1-14,1 thousand years) created a lake 110 meters deep during maximal expansion (Argollo and Mourguiart, 2000; Placzek et al., 2009) that covered a maximum area of 60,000 km^2 (including the totality of the Uyuni SF, Fig. 1c). The second event corresponds to the Coipasa cycle (12.8-11.4 thousand years) that reached the maximum depth of ~60 meters (Placzek et al., 2011). The third and last large flood event occurred during the period 1984-1986 (Roche et al., 1991;

Zolá and Bengtsson, 2007; Senamhi, 2011). These three flood events have created lakes of different depths, which in turn inundated the islands at different heights with respect to elevation (Fig. 1).

At present it is possible to observe four stages with different soil ages in several of the islands of the Uyuni-SF archipelago (Fig. 1), which together represent a system of vertical chronosequences, with some islands showing (depending on height) a long ca. 20,000-years succession with four stages of development associated with flood events (Fig 1c). The slopes in all stages of development within islands are similar, because of the pyramidal structure of islands. Since these islands have similar history of disturbance, climate and biodiversity and only differ in parent material (Sedimentary, Volcanic-1 and Volcanic-2), they provide an unparalleled opportunity to assess the major drivers of soil development within islands (i.e., across time within a chronosequence) and among islands (i.e. comparing similar ages among chronosequences differing in parent material).

Study site

Across the Andean Puna annual precipitation is concentrated in austral summer months with 80% of the rainfall occurring from December to February (Vuille et al., 2000) and mean annual precipitation for the last 40 years is 176 ± mm. Average annual temperature is 8.5°C, with freezing temperatures common in seven months of year (~200 days) and maximum temperatures (~23 °C) registered during summer season (data from Meteorology and Hydrology National Service, Bolivia). We selected three chronosequences, which can be assigned into three categories related to the parent material (sedimentary, volcanic-1 and volcanic-2) (Fig. 1). Each chronosequence is ca. 800 m long spanning four successional stages and the following biotic and abiotic characteristics:

• Stage 1. This stage is on average 25 years-old and across chronosequences has sandy soils, low diversity of perennial and annual plants, and relatively low shrub. The dominant species are: *Atriplex imbricata, Baccharis tola, B. boliviensis* and *Chuquiraga atacamensis.*

• Stage 2. 11,400 years-old. This stage has highshrub cover, with abundant individuals. The dominant shrub species are: *B. boliviensis, B. tola, Krameria sp* and *Junellia seriphioides*.

• Stage 3. 14,100 years-old. This stage represents a typical shrubland with high diversity and cover of deciduous shrubs. The dominant species are: *Senecio phylloleptus*, *Trichocereus atacamensis* and *Lycium chanar*.

• Stage 4. 20,000 to 21,000 years-old. This stage represents the top of sequence, and is characterized by low biomass production and scattered vegetation. The dominant species are annual plants: *Euphorbia ovalifolia* and *Tagetes multiflora*.

Sampling

At all three chronosequences, we set up four plots (30 x 2 m) at each of the four stages of ecosystem development. All plots were arranged with a west-facing slope aspect. Soil and plant samples were collected at the end of the rainy season (early May) when primary productivity is highest. At each plot, we collected soils from the 0-10 cm layer in three randomly assigned points; these subsamples were pooled and homogenized into a single sample, and stored in plastic bags at -4°C before laboratory analysis. Soil pH was determined on 5g sample in both 20 ml of distilled water and 0.01 mm $CaCl_2$ using a glass electrode. Total soil C and N contents were estimated by flash combustion in a Carlo Erba NA 2500 elemental analyzer. Phosphorus (P) was extracted with a concentrated sulfuric acid water peroxide solution in a Hach Digesdahl digestor and determined by the molybdenum-blue method (Steubing and Fangmeier, 1992). Soil moisture (g H_2O/g soil)

was estimated by calculating the difference between fresh and oven-dried weight (48 h at 70 °C). All biogeochemical analyses were carried at the Biogeochemistry Laboratory, Department of Ecology, Pontificia Universidad Católica de Chile.

Plant species

Percentage of annual and perennial plant cover were recorded on each plot. Plant cover was determined by point-intercept method (Jonasson, 1988) and plant species were collected and identified to highest taxonomic level. Simultaneously, young-mature leaves and litter were collected from 20 individuals of two shrub species that occur in all stages the three chronosequences. We chose the dominant deciduous (*Atriplex imbricata*) and evergreen (*Baccharis tola*), these two shrubs represent 50% of total plant cover in all chronosequences. Henceforth refer to by genus only. The halophyte *Atriplex* is a deep-rooted perennial shrub that replaces its small and abundant leaf during the dry season (May to August), while *Baccharis* is a perennial shrub with abundant and resinous leaves. Recently fallen leaf litter was collected from the same individuals. Leaf samples were dried at 50 °C for 48 h and used for N and P concentrations analysis. Nutrient concentrations in leaf and litter were used to calculate resorption efficiency and proficiency (Killingbeck, 1996). Nutrient resorption efficiency is the proportion of nutrients resorbed from fresh leaf prior to leaf fall. Resorption proficiency is the nutrient concentration in senescent leaves (*sensu* Killingbeck, 1996).

Decomposition

To assess differences in litter decomposition across stages of soil development, we conducted a below and aboveground litter decomposition experiment in the field. Belowground experiments were conducted on cellulose filters, Whatman®, of 1.72 g buried at 10 cm depth in plastic mesh-bags and left to decompose for the nine dry months (March to December). At the end of the dry season, just before the first rains, we collected all samples and took them to the lab for dry weight measurements. We used cellulose filters with identical chemistry (98% cellulose) in all stages and chronosequences because our main goal was to determine the effect of soil properties (e.g. nutrient content) on decomposition across soil development without the confounding effect of litter quality.

The aboveground decomposition experiment was carried out to evaluate litter decomposition of *Atriplex imbricata*. This experiment was performed only in sedimentary and volcanic-2 chronosequences as an extraordinary climatic events render impossible to have access to the volcanic-1 chronosequence to recovery litter samples after the experiment. Fresh litter (5 g) of *Atriplex* from four stages across soil development was placed in litterbags (20 x 20 cm) and exposed to solar radiation during seven months (May to December). After this period remnant litter was collected and weight loss was recorded. Litter nutrient (C, N and P) and lignin concentrations were analyzed before and after of experiment.

Data analysis

We performed a one-way analysis of variance (ANOVA) to examine differences in biotic (plant cover) and abiotic (soil properties) among stages of soil development. Significance levels were set at $\alpha = 0.05$ for all ANOVAs, post-hoc comparisons among stages were

performed using Tukey's HSD test. Differences in mean soil and plant characteristics among chronosequences were determined using a Generalized Lineal Model Nested design, with stage as a fixed effect nested within chronosequence origin. Changes in leaf and litter lignin, weight and nutrient concentrations were evaluated using one-way ANOVA. All data were tested for homogeneity of variances and standardized if necessary to meet ANOVA assumptions. All nutrient ratios were corrected with atomic weight before analysis. All mean values are shown with ±1SE. The effect of soil properties on plant cover and decomposition changes across soil development was evaluated by multiple regressions. Analyses were conducted in Statistica 9.0 (StatSoft Inc., Tulsa, Oklahoma, USA) and R (vegan) ver. 2.9.1 (Development Core Team 2009).

RESULTS

Soil properties in the Uyuni-SF

Soil properties were different among chronosequences and in particular between sedimentary and volcanic-2 chronosequences. Out of the nine soil factors evaluated eight were significantly different among chronosequences (Table 2). In the sedimentary chronosequence soil nutrient pools (i.e. total-N, P and P_{OLSEN}) were significantly lower compared to other two chronosequences, while soil pH, soil salinity and total C were higher (Table 2).

Soil nutrients, N:P and soil pH patterns across soil development

Across the Uyuni-SF soil N:P was lower in all chronosequences, and these patterns were determined by low soil N (Fig. 2a). Among chronosequences, the sedimentary ones showed the lower values of soil N, soil P and soil N:P (Fig. 2b, 2c). Soil N:P increased along soil

age gradient in all chronosequences (Fig. 3). In volcanic-1 and volcanic-2 chronosequences this pattern was determined by increases in soil N from young to old stages, as soil P remained constant across all stages of development. In sedimentary chronosequence, however, soil P did decline with soil age (Table 2). Lowest soil N:P were recorded in the youngest soils of all chronosequences (Fig. 3d), but around of 11,000 years (i.e. the second stage) N:P values in the sedimentary chronosequence remained low, whereas in the volcanic-2 and volcanic-1 chronosequences soil N:P increases in 300 and 400 %, respectively. Contrary to soil N:P, soil pH declined across soil development in all chronosequences (Fig. 3c), with late stages of development showing the lowest values of soil pH in the three chronosequences. However, soil pH decrease in sedimentary chronosequence was less pronounced than other two chronosequences, indeed, all soils across soil development in sedimentary chronosequence are in the category of alkaline soils, with values over eight.

Ecosystem processes across soil development

Plant productivity

Changes in soil N:P and soil pH across soil development were accompanied by changes in plant cover (%), and decomposition rates in all chronosequences (Fig. 3). Following a rapid increase in intermediate stages, plant cover declined during late stages of development in all chronosequences (Fig. 3b). Plant cover values in late stages of development were about half of maximal plant cover reached around of 11,000 years (i.e. second stage) in all chronosequences. Multiple regression analysis showed that plant cover change during soil development was determined mainly by soil pH in volcanic-1 (R^2= 0.46; p< 0.01) and volcanic-2 chronosequences (R^2= 0.46; p< 0.01), meanwhile, in the sedimentary

chronosequence declines in plant cover during late stages of development were determined by changes in soil N:P, soil pH and soil salinity (R^2= 0.72; p< 0.01; Table 3). Since the deciduous *Atriplex* and the evergreen *Baccharis* represented 54% of total plant cover in all chronosequences (Table 2), the observed unimodal pattern of plant cover across soil development on three chronosequences was largely determined by changes in cover of these two shrub species, in particular by large increase of *Atriplex* during second stages of development.

Leaf and litter nutrient content across soil development

Leaf and litter Nitrogen contents

Fresh leaf-N in *Atriplex* increased across soil development in all chronosequences (Fig. 4), with higher values observed in the latest stages of soil development. In terms of litter-N, in contrast, we found increases across soil development in sedimentary (Fig. 4a) and volcanic-1 chronosequences (Fig. 4c), but not in the volcanic-2 chronosequence (Fig. 4e).

Fresh leaf-N in *Baccharis* also increased with soil age in sedimentary and volcanic-1 chronosequences (Fig. 4b, 4d), and this pattern was not observed in the volcanic-2 chronosequence (Fig. 4e, 4f). In terms of litter-N, plants from youngest stages were below the concentration that indicates that resorption proficiency was complete (*sensu* Killingbeck, 1996). This pattern was observed in the three chronosequences, thus these results suggest, not only that soils are poor in N, but also that plant have strategies to increase nutrient retention.

Leaf and litter Phosphorous contents

In *Atriplex* leaf-P decreased across soil development in the volcanic-1 chronosequence (Fig. S1a), with higher values after 11,000 years (i.e. second stage of development) meanwhile, in sedimentary and volcanic-2 chronosequences leaf-P not changed significantly across soil development. Litter-P, decreased in sedimentary and volcanic-1 chronosequences (Fig. S1a, S1c) while in volcanic-2 chronosequence it remained unchanged across soil development. Litter P concentrations were above the maximum value of resorption proficiency in all stages of development in all chronosequences (Fig. S1), indicating that P resorption from litter before leaf abscission was low in all chronosequences (*sensu* Killingbeck, 1996).

Leaf-P in *Baccharis* increased across soil development in the volcanic-1 chronosequence, and declined in the sedimentary chronosequence (Fig. S1). Litter P, on the other hand, declined across soil age gradient in sedimentary ($F_{3,12}$= 9.15; p< 0.01) and volcanic-2 chronosequence ($F_{3,12}$= 5.89; p= 0.01). Resorption proficiency was higher in litter from late stages of development in the sedimentary chronosequence, than litter from intermediate stages in volcanic-2 chronosequence.

N and P resorption efficiency

In contrast to expectations N resorption efficiency in *Atriplex* tended to increase with soil age in volcanic-1 and volcanic-2 chronosequences (Fig.5c, 5e), however, increases only were significant in the volcanic-1 chronosequence ($F_{3,12}$= 9.69; p= 0.02). In the sedimentary chronosequence, N and P resorption did not show increases with soil development (Fig. 5a). In *Baccharis*, as expected, N resorption efficiency declined with soil in volcanic-1 ($F_{3,12}$= 3.74; p= 0.04; Fig. 5d) and volcanic-2 chronosequences ($F_{3,12}$= 3.48; p= 0.05; Fig. 5f). While, in the sedimentary chronosequence, and contrary to our expectations N

resorption efficiency increased across stages of development, although this pattern was not significant (Fig.5b).

Soil N:P and leaf and litter N:P relationships

Changes in soil nutrient pools were poor predictors of changes in green leaf and litter nutrient contents during soil development in all type of chronosequences (Fig. 6). However, when nutrient ratios from soil and plant were evaluated we found that increases in litter N:P of *Atriplex* across soil development in the sedimentary chronosequence was related to increases in soil N:P (Fig. 6a). In *Baccharis* litter N:P increased with soil N:P across soil development (Fig. 6d). Contrary to our expectation, leaf and litter N:P of *Atriplex* from the volcanic-2 chronosequence was negatively related to increases in soil N:P during soil development. In the volcanic-1 chronosequence soil N:P increase and leaf and litter N:P were positively related, however, these relationships were not significant.

Decomposition

Aboveground decomposition experiments showed that litter decomposition (expressed as weight loss) during seven months was higher in late stage of development in the sedimentary chronosequence (Fig. 7a). Highest decomposition was observed in the late stage of development where litter C:N was lower (Fig. 7a). In contrast, in the volcanic-2 chronosequence decomposition was not significantly related to soil age (Fig. 7a). This is may be the product of the fact that litter C:N remained constant along soil development in the volcanic-2 chronosequence (Fig. 7e).

Belowground decomposition expressed as percentage mass loss increased significantly across soil development in the three chronosequences (Fig. 7). Paper filters in

late stages of development showed the highest amount of decomposition which was twice as much than in early stages in all chronosequences (Fig 7b-7d). Soil pH explained 60% and 51% of deviance in filters decomposition in volcanic-1 and volcanic-2 chronosequences respectively, meanwhile, in the sedimentary chronosequence the observed increases in decomposition during late stages of development, was not significantly related to changes in soil factors (Table 4).

DISCUSSION

N and not P limitation across soil development

Slow and imperceptible decline in soil P after 20,000 years of soil development contrast with patterns reported in chronosequences from mesic environments (Wardle et al., 2004; Parfitt et al., 2005) where P limitation begins rapidly after 5,000 years (Richardson et al, 2004) or 7,000 years (Laliberte et al., 2012). In fact, soil P values in oldest stages in our three chronosequences (*ca* 20,000 years) were relatively higher than early stages of development in chronosequences from Australia (Laliberte et al., 2012), and similar to young stages of 130-280 years in a chronosequence from New Zealand (Richardson et al., 2004). In contrast, soil N and N:P ratio in the Uyuni-SF was lower than any of six chronosequences from distinct zones of the planet reported in Wardle et al, (2004). Indeed, values of soil N:P in late stage of development in our three chronosequences were even lower than youngest stages of those six chronosequences. While evidence from distinct chronosequences suggest that soil N typically increase through early successional stages and after decline or not vary with soil age (Richardson et al., 2004; Wardle et al., 2004; Jangid et al., 2013), in our system soil N increased across soil development in all

chronosequences, and this determined that soil N:Pincreased during late successional stages.

Transition from N to P limitation across soil development has been recorded in chronosequences worldwide (Wardle et al., 2004), thereby, this pattern is considered as a regular feature of long-term soil development (Peltzer et al., 2010). However, considering that N or P limitation is largely a consequence of balance between losses and inputs during soil development (Vitousek et al., 2002b; Vitousek et al., 2010), it is feasible that this transition never occurs (Menge et al., 2012), in particular, in ecosystems inherently N or P limited where specific nutrient limitation could expand across all stages of soil development. For example, rapid P limitation early during soil development was a consequence of increases in soil N by microbial N-fixers activity that enhance N:P ratio (Richardson et al., 2004). In the Uyuni-SF soil properties after *ca*. 20,000 years of development strongly suggests that N limitation spreads across all stages of soil age gradient. We suggest three plausible explanations for this pattern. First, high inorganic N losses relative to P losses via rapid volatilization because of low atmospheric pressure and low vegetation cover, similar to data reported in arid ecosystems (Evans and Johansen, 1999; Vitousek et al., 2002; Yahdjian and Sala, 2010). Indeed, the combination of high elevation and dry conditions in the Uyuni-SF could increase losses by volatilization. Second, low microbial N input determined by low temperature that increases the energetic cost for Nitrogenase activity (Houlton et al., 2008) and alkaline soils that reduce microbial function (Rousk et al., 2009; 2010) could contribute to low N availability on Andean soils. In part, highly alkaline soils could substantially inhibit nodulation by reducing soil and plant colonization by N-fixing bacteria (Bordeleau and Prevost, 1994; Belnap, 2001), and increasing costs for N-fixation might reduce the competitive advantage of symbiotic

bacterial-plant associations (Houlton et al., 2008). This suggestion coincide with low diversity of N-fixing plants observed in the Uyuni-SF, with only 3 species (~5 % of all species) that were recorded occurring mostly on early stages of development. Third, intense changes in climatic conditions during last 20.000 years that determined prolonged floods events could increases hydrologic losses of organic N, similar to those observed in mesic temperate forest where high precipitation increases dissolved organic N losses (Hedin et al., 1995; Perakis and Hedin, 2002). These processes could help to explain the increases of soil N across soil age gradient observed in our chronosequences. Therefore, slow soil development in the Uyuni-SF chronosequences might be determined by interaction between high N losses relative to P losses and extreme low N inputs from relatively poor microbial N-fixers communities, all this directly related to actual and past climate conditions that together would contribute to extend N limitation indefinitely, keeping the system in an eternal unproductive youth.

Parent material effect

In the Uyuni-SF soil development in the sedimentary chronosequence was slower than that observed in the volcanic-1 and volcanic-2. Changes in soil properties across soil development strongly suggest that all stages (independently of soil age) in sedimentary chronosequence are in an initial stage of development. For example, soil pH in all stages of sedimentary succession was above 8.04 that represent the mean of soil pH in the Uyuni-SF. Soil pH generally tends to decline rapidly as soil age increase across primary and secondary successions (Huggett, 1998; Rhoades et al., 2008; Laliberte et al., 2012), in particular on late successional stages where soils tend to be highly acidic (pH < 5) and associated with low decomposition rates (Peltzer et al., 2010). Likewise, soil N:P differences among

sedimentary and volcanic-2 chronosequences were largely determined by higher soil N content across soil development in volcanic-2 chronosequence. Soil N in third and fourth successional stages of volcanic-1 and volcanic-2 chronosequences was two-fold of same stages in sedimentary chronosequence.

Since multiple environmental factors might affect the development of soils across long-term successions (Anderson, 1988; Hugget, 1998), natural experiments as the one presented in this study, could improve significantly our ability to detect major drivers of soil development across millennial time scale (Wardle et al., 2012). In particular because large differences observed in soil properties across soil development among chronosequences in the Uyuni-SF, indicates that soils developing under the same environmental conditions (i.e., climatic) could show different rates of development. We suggest two plausible effects associated to the different geologic origin of chronosequences to explain these patters. First, because transformation of parent material into soil requires prolonged interaction of abiotic (e.g. weathering) and biotic (e.g. microbial mineralization) processes (Hugget, 1998), the type and geological origin of parent material could affect the rates of substrate-derived nutrients release during soil development (Yavitt, 2000). In particular, soil P availability could be different among soils derived from distinct parent material (Walker and Syers, 1976; Vitousek et al., 2010), and more importantly the rate of nutrient releases across soil development could be largely different among volcanic and sedimentary soils (Anderson, 1988). Soils derived from volcanic parent material often contain large amounts of allophane (Escudey et al., 2001; Lilienfein et al., 2003) that reduce drastically P availability by rapid phosphate absorption and retention with amorphous Aluminum (Parfitt, 1980; Borie and Zunino, 1983). These processes characteristics of volcanic soils are associated to strong immobilization of organic matter

and formation of compounds such as Al-phosphate and Fe-phosphate difficult to access by plants and microorganisms (Lilienfein et al., 2003; Parfitt et al., 2005). These differences in soil P availability in soils from distinct parent material could modify early biological activity during soil development, thereby also effect soil processes such as N-fixation (Wardle et al., 2003; Vitousek et al., 2010). Second, soil pH is a critical factor to soil biological process across long-term soil development (Richardson et al., 2004; Laliberte et al., 2012) through its effects on reaction capacity and availability of some essential nutrients (e.g. N, P) (Kemmitt et al., 2006; Aciego Pietri and Brookes, 2008). Large differences in soil pH among soils with different geological origin have been reported. For example, volcanic Andesitic rocks (similar to volcanic chronosequences in the Uyuni-SF) often have acidic soils, with pH ranging between 5 and 6.5 (Lilienfeinet al., 2003; Rasmussen et al., 2007), meanwhile, sedimentary soils from semiarid environments frequently have alkaline to moderately alkaline soils and associated to high availability of Calcium and Carbonates (Bordeleau and Prevost, 1994; Delgado-Baquerizo et al., 2013). Extreme variations in soil pH in the Uyuni-SF could help us to explain differences in soil properties among stages and among chronosequences with soils derived from distinct parent material. N-transformation rates and microbial N-fixation are largely dependent of soil pH (Bordeleau and Prevost, 1994; Rousk et al., 2009). Because processes such as nitrification and mineralization tend to be higher in neutral soils (pH ~7) (Delgado-Baquerizo et al., 2013), soil N availability increases around 7-7.5 of pH, declining strongly in alkaline soils (Boot et al., 2005). Thereby, soil pH variation has a strong control on inputs and turnover of N forms during soil development. Among chronosequences in the Uyuni-SF different rates of soil N increases during soil development could be explained by differences in soil pH across soil age gradient, particularly given that extremes alkaline

soils in sedimentary chronosequence were associated to lower N content, meanwhile the volcanic-2 chronosequence with moderately alkaline soils showed higher N content across most stages of development.

Slow soil development drives ecosystem development

Recent reviews of long-term soil development emphasize that geochemical change during pedogenesis through their effects on some critical ecosystem processes such as primary productivity, decomposition and nutrient cycling; enables to identify two contrasting phases of ecosystem development (Wardle et al., 2004; Peltzer et al., 2010; Laliberte et al., 2013). The first called "ecosystem progressive phase" is characterized by increasing biomass production, high rate of decomposition and soil nutrient availability, which depending on climate conditions could extended for several thousands of years. The second period is characterized by a substantial decline in plant biomass associated to reduced decomposition and nutrient cycling rates and is called "retrogressive phase" (*sensu* Wardle et al., 2004). Therefore, maximal plant biomass reached during intermediate stages of soil development tends to decline as soil P decrease during late successional stages (Wardle et al., 2004). In all chronosequences from the Uyuni-SF plant cover showed a hump-shaped pattern, with maximal values recorded in intermediate stages. Unimodal patterns in plant cover have also been shown by Wardle et al, (2004) and are, related to changes in soil N:P during soil development in our sedimentary chronosequence. Plant cover declining in late stages of development was not determined by P limitation. Instead these patterns in the Uyuni-SF were determined by changes in relative abundance of dominant shrub species, with halophyte *Atriplex* dominating in early stages of development and declining during late stages of development.

On the other hand, as expected, belowground decomposition increased across soil development, and these patterns were largely associated to changes in soil pH. During late stages of development decomposition was higher and soil pH was close to neutral (~7). Because decomposition rates are determined by soil microbial activity (Schlesinger and Hasey, 1981; Van der Heijden et al., 2008), in particular composition and biomass of bacteria and fungi communities (Fierer et al., 2009), we suggest that the observed improve in soil pH conditions as soil age increased could favor microbial activity, increasing decomposition. In particular, because microbial metabolic activity is highly dependent of soil conditions such as soil nutrient availability (notably N and P) and soil pH (Gallardo and Schlesinger, 1992; Fierer et al., 2009). These results were corroborated when microbial biomass was evaluated across soil development, showing that bacterial biomass increased significantly across soil age gradient (Alfaro et al, Chapter 3).

Soil development and plant nutrient resorption

Low litter N:P in both types of shrubs in all chronosequences was mainly determined by high N resorption proficiency. However the magnitude of N resorption was largely different among chronosequences. For example, in the sedimentary chronosequence the 93% (evergreen) and 86% (deciduous) of plant sampled had litter N under of 0.07; showing high resorption proficiency (*sensu* Killingbeck, 1996) which is indicative of strong N limitation (Richardson et al., 2005). While in volcanic-1 and volcanic-2 chronosequences these values fluctuated around of 55 %. This pattern could be also reflecting differences in soil N content among chronosequences, in particular because soil N was lower in the sedimentary chronosequence. Such reduced nutrient content in litter due to large resorption might have strong effects on litter decomposition and N recycling (Mueller et al., 2012). In

contrast, P resorption proficiency (under 0.05) was relatively low in all chronosequences, in particular in deciduous plants, suggesting that P is not a limiting nutrient in the Uyuni-SF. At difference of chronosequences from mesic and subtropical environments N resorption proficiency was higher only in early stages of development (Crews et al., 1995; Richardson et al, 2004; Wardle et al, 2004). Leaf and litter chemistry of deciduous and evergreen species showed strong shifts across soil development in all chronosequences. These intraspecific changes in N and P concentrations can be mainly due to phenotypic plasticity across soil age gradient (Chapman et al., 2006). Although leaf and litter N:P increased across soil development in all chronosequences, leaf and litter chemistry was not significantly related to increase in soil N:P in all chronosequences.

Has been widely reported that deciduous plants contain higher concentration of N and P in leaf and litter than do evergreen species (Aerts, 1995), therefore both groups would have different impacts on soil nutrient availability because differential degradability of litter (Mueller et al., 2012). Across soil development in all chronosequences, these differences were short and marginally significant. Variability in leaf and litter nutrient content among individual plants of evergreen shrubs was lower than do deciduous shrubs in all chronosequences. Indeed, similar N resorption patterns in shrubs species (deciduous and evergreen) during early stages of soil development in sedimentary and volcanic-1 chronosequences could have important effects on litter decomposition and N recycling on stages lower with soil N content (Aerts, 1995; Chapman et al., 2006).

N-limitation and soil development in dryland mountain environments

Because N cycling ultimately depends on the balance between inputs and outputs, and the relative rates at which N cycle among different components (i.e. soil and plants); the rate of

soil development during early stages will depend of ability of biotic components (i.e. microbial N-fixing) to increase the input of atmospheric N and accelerate organic mineralization and decomposition (Vitousek et al., 2002). However, the geographic distribution and taxonomic diversity of organisms with biological N-fixation capacity is largely determined by environmental conditions (Sprent and Sprent, 1990; Cleveland et al., 1999), such as temperature, humidity, soil properties (e.g. pH, salinity) and UV radiation (Vitousek et al., 2002b). In dryland mountain environments where combination of permanently water limitation and low mean temperature reduce the diversity and activity of free-living heterotrophic N-fixing bacteria (Hooper and Johnson, 1999; Vitousek et al., 2002) and symbiotic bacterial communities (Houlton et al., 2008); low biological N-fixation could significantly hinder soil development (Körner, 1989; Cleveland et al., 1999). The response of aboveground organisms to this N-limitation could even increase limitation by the dominance of species with low nutrient content (Muller et al., 2012) and that produce low quality litter (N:lignine), reducing significantly the decomposition.

PERSPECTIVES, CAVEATS & CONCLUSIONS

Overall, we suggest that slow soil development and associated low soil N content in our system is best explained by dry and cold conditions that reduce N fixation by increasing the energetic cost for Nitrogenase activity (Houlton et al., 2008). Low diversity of plant N-fixing species across the Uyuni-SF and within chronosequences (~5 % of total species) and lower microbial activity (N-fixing bacteria) suggest that N input on this system could be less than expected, and reinforce the general idea that environments in Andean Dry Puna are extremely sensitive to disturbance. This observation is critical for understanding the effect of disturbance on soil and ecosystem development. In particular, because in the last

15 years it has been emphasized the relative importance of disturbances to rejuvenate old and P-limited soils in mesic environments. However, our results indicate that catastrophic disturbances in this N-limited ecosystem could have strong negative effects on soil development, because, recovery after disturbances could extend for long-time.

Even our chronosequences have only four stages of soil development, the patterns above and belowground strongly suggest that ecosystem process such as plant production, decomposition and nutrient resorption were highly coupled to increases in soil N and reduction in soil stressors such as soil pH and salinity. This last result is critical because highlights the additional indirect effects of disturbances on soil development. In particular, because highly alkaline and saline soils in the early stages were shifting to more neutral and less saline soils as soil development proceeds.

The effect of parent material on soil properties across long-term soil development never has been evaluated. However, our results point out to substantial effect of geological origin of parent material on rate of soil development and availability of soil nutrients, and claim to explicit incorporation to this factor on models evaluating long-term soil development.

ACKNOWLEDGEMENTS

We thank to Paola Villarroel, Daniela Rivera, Huber Villca, Alejandro Coca and Mauricio Torrico for helping with field and lab work. We are very grateful with Center of Biodiversity and Genetics (CBG) for logistic support for fieldwork. F. D. Alfaro was funded by a doctoral scholarship from CONICYT AT-24100099 and Russell E. Train Education for Nature Fellowships. This project was funded by ICM P05-002 and CONICYT PFB-023.

LITERATURE CITED

Aciego Pietri, J. C., andP. C. Brookes. 2008. Nitrogen mineralisation along a pH gradient of a silty loam UK soil. Soil Biology and Biochemistry 40:797-802.

Aerts, R. 1995. The advantages of being evergreen. Trends in Ecology & Evolution 10:402-407.

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: are there general patterns?. Journal of Ecology 84:597-608.

Alfaro, F. D., A. Gaxiola, P. A. Marquet. Soil bacterial and fungal communities display contrasting patterns across long-term ecosystem development: evidence from Andean Dry Puna. In press.

Anderson, D. W. 1988. The effect of parent material and soil development on nutrient cycling in temperate ecosystems. Biogeochemistry 5:71-97.

Andrade, M. O., P. Ergueta and M. V. Sanjines.2006. Conservación y desarrollo sostenible en el suroeste de Potosí, Bolivia. Prefectura del Departamento de Potosí.

Argollo, J., and P. Mourguiart. 2000. Late Quaternary climate history of the Bolivian Altiplano. Quaternary International 72:37–51.

Asociación Boliviana para la Conservación (ABC). 2001. Evaluación ecológica de las formaciones de islas ubicadas al interior del Salar de Uyuni. Informe final. La Paz, Bolivia.

Austin, A. T. 2011. Has water limited our imagination for aridland biogeochemistry? Trends in Ecology &Evolution 26:229–35.

Baied, C. A., and J. C. Wheeler. 2013. Evolution of high Andean Puna ecosystems: Environment, climate, and culture change over the last 12000 years in the central Andes.Mountain Research and Development 13:145–156.

Baker, P.A., et al. 2001. Tropical climate changes at millennial and orbital timescales on the Bolivian Altiplano. Nature 409:698–701.

Bannert, A., et al. 2011. Changes in diversity and functional gene abundances of microbial communities involved in nitrogen fixation, nitrification, and denitrification in a tidal wetland versus paddy soils cultivated for different time periods. Applied and Environmental Microbiology 77:6109-6116.

Baron, J. S., D. S. Ojima, E. A. Holland, and W. J. Parton. 1994. Analysis of nitrogen saturation potential in Rocky Mountain tundra and forest: implications for aquatic systems. Biogeochemistry 27:61-82.

Belnap, J. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. Biological soil crusts: structure, function, and management.241-261.

Booth, M. S., Stark, J. M., and E. Rastetter. 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs 75:139-157.

Bordeleau, L. M., and D. Prévost. 1994. Nodulation and nitrogen fixation in extreme environments. In Symbiotic Nitrogen Fixation (pp. 115-125).Springer Netherlands.

Borie, F., and H. Zunino. 1983. Organic matter-phosphorus associations as a sink in P-fixation processes in allophanic soils of Chile. Soil Biology and Biochemistry 15:599-603.

Cardenás, J., and W. Choque.2008. Fertilidad, uso y manejo de suelos en la zona del intersalar, departamentos de Oruro y Potosí. Programa Quinua Altiplano Sur. Fundación AUTAPO, Universidad Técnica de Oruro, Prefectura de Oruro, Oruro

Carreira, J. A., and K. Lajtha. 1997. Factors affecting phosphate sorption along a Mediterranean, dolomitic soil and vegetation chronosequence. European Journal of

Soil Science 48:139-149.

Carreira, J. A., Lajtha, K., and F. X.Niell. 1997. Phosphorus transformations along a soil/vegetation series of fire-prone, dolomitic, semi-arid shrublands of southern Spain Soil P and Mediterranean shrubland dynamic. Biogeochemistry 39:87-120.

Cary, R. S., and W. Angulo.2006. Efecto del descanso agrícola sobre la microbiota del suelo (Patarani - Altiplano Central boliviano Altiplano). Ecología en Bolivia 41:103–115.

Chadwick, O. A., et al. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature 397:491-497.

Chadwick, O. A., and J. Chorover. 2001. The chemistry of pedogenic thresholds. Geoderma 100:321-353.

Chapman, S. K., T. G. Whitham, and M. Powell. 2006. Herbivory differentially alters plant litter dynamics of evergreen and deciduous trees. Oikos 114:566-574.

Cleveland, C. C., et al. 1999. Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. Global Biogeochemical Cycles 13:623-645.

Crews, T. E., et al. 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. Ecology 76:1407-1424.

Delgado-Baquerizo M., et al. 2013. Aridity Modulates N Availability in Arid and Semiarid Mediterranean Grasslands. PLoS One 8:59807-59814.

Deubel.A., W. Merbach. 2005. Influence of Microorganisms on Phosphorus Bioavailability in Soils. In: F. Buscot and A. Varma (eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag, Berlin Heidelberg, Germany.

Drewnik, M. 2006. The effect of environmental conditions on the decomposition rate of

cellulose in mountain soils. Geoderma 132:116–130.

Escudero, A., J. M. Del Arco, I. C. Sanz, and J. Ayala. 1992. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. Oecologia 90:80-87.

Escudey, M., et al. 2001. Chemical forms of phosphorus of volcanic ash-derived soils in Chile. Communications in Soil Science and Plant Analysis 32:601-616.

Esperschütz, J., A. et al. 2011. Microbial food web dynamics along a soil chronosequence of a glacier forefield. Biogeosciences 8:1275-1308.

Evans, R. D., and J. R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from δp15N of soils. Oecologia 94:314-317.

Evans, R.D., and J. R. Johansen. 1999. Microbiotic crusts and ecosystem processes. Critical Reviews in PlantScience 18:183–225.

FAO, Mountain Partnership Secretariat, UNCCD, SDC, CDE. 2011. Highlands and Drylands – mountains, a source of resilience in arid regions. Published by FAO, UNCCD, Mountain Partnership, Swiss Agency for Development and Cooperation, and CDE, with the support of an international group of experts. Rome.

Fenn, M. E., et al. 2003. Ecological effects of nitrogen deposition in the western United States.BioScience 53:404-420.

Fierer, N., et al. 2009. Global patterns in belowground communities. Ecology Letters 12:1238-1249.

Fornara, D. A., and D. Tilman. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology 96:314-322.

Gallardo, A., and W. H. Schlesinger. 1992. Carbon and nitrogen limitations of soil microbial biomass in desert ecosystems. Biogeochemistry 18:1-17.

Gallardo, M. B., Perez, C., Nuñez-Avila, M., and J. J. Armesto. 2012. Desacoplamiento del desarrollo del suelo y la sucesión vegetal a lo largo de una cronosecuencia de 60 mil años en el volcán Llaima, Chile. Revista Chilena de Historia Natural85:29-306.

Galloway, J. N., et al. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889-892.

Gaxiola, A., McNeill, S. M., and D. A. Coomes. 2010. What drives retrogressive succession? Plant strategies to tolerate infertile and poorly drained soils. Functional Ecology 24:714-722.

Hedin, L. O., Armesto, J. J., A. H. Johnson. 1995. Patterns of nutrient loss from unpolluted, old-growth temperate forests: evaluation of biogeochemical theory. Ecology 76:493-509.

Hooper, D. U., and L. Johnson. 1999. Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. Biogeochemistry 46:247-293.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–30.

Huggett, R. 1998. Soil chronosequences, soil development, and soil evolution: a critical review. Catena 32:155–172.

Jangid, K., W. B. Whitman, L. M. Condron, B. L. Turner, and M. A. Williams. 2013. Progressive and retrogressive ecosystem development coincides with soil bacterial community change in a dune system under lowland temperate rainforest in New Zealand. Plant and Soil 367:235–247.

Jonasson, S. 1988. Evaluation of the point intercept method for the estimation of plant biomass. Oikos 52:101-106.

Kemmitt, S. J., Wright, D., Goulding, K. W., and D. L. Jones. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biochemistry 38:898-911.

Killingbeck, K. T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology. 77: 1716-1727.

Körner, C. 1989. The nutritional status of plants from high altitudes. A Worldwide Comparison.Oecologia. 81: 379-391.

Lajtha, K., and W. H. Schlesinger. 1988. The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. Ecology 69:24-39.

Laliberté, E., et al. 2012. Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. Journal of Ecology 100:631–642.

Lilienfein, J., Qualls, R. G., Uselman, S. M., andS. D. Bridgham. 2003. Soil formation and organic matter accretion in a young andesitic chronosequence at Mt. Shasta, California. Geoderma 116:249-264.

Latorre, C., J. L. Betancourt, and M. T. K. Arroyo. 2006. Late Quaternary vegetation and climate history of a perennial river canyon in the Río Salado basin (22°S) of Northern Chile. Quaternary Research 65:450–466.

Lichter, J. 1998. Rates of weathering and chemical depletion in soils across a chronosequence of Lake Michigan sand dunes. Geoderma 85:255-282.

Menge, D. N., Hedin, L. O., and S. W. Pacala.2012. Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. PloS One 7:42045-42071.

Mueller, K. E., S. E. Hobbie, J. Oleksyn, P. B. Reich, and D. M. Eissenstat. 2012. Do

evergreen and deciduous trees have different effects on net N mineralization in soil?. Ecology 93:1463-1472.

Navarro, G., and W. Ferreira. 2004. Zonas de vegetación potencial de Bolivia: Una base para el análisis de vacíos de conservación. Revista Boliviana de Ecología y Conservación Ambiental 15:1-40.

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. Annual Review of Ecology and Systematics 4:25-51.

Parfitt, R. L. 1980. Chemical properties of variablecharge soils. In: Soils with variable charge (ed. B.K.G. Theng) pp 167-194. New Zealand Soil Bureau, Lower Hutt, New Zealand.

Parfitt, R. L., et al. 2005. N and P in New Zealand soil chronosequences and relationships withfoliar N and P. Biogeochemistry75:305-328.

Peltzer, D. A., et al. 2010. Understanding ecosystem retrogression. Ecological Monographs 80:509-529.

Perakis, S. S., and L. O. Hedin. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature 415:416-419.

Placzek, C. J., J. Quade, and P. J. Patchett. 2011. Isotopic tracers of paleohydrologic change in large lakes of the Bolivian Altiplano. Quaternary Research 75:231–244.

Placzek, C., et al. 2009. Climate in the dry central Andes over geological, millennial, and interannual timescales. Annals of the Missouri Botanical Garden 96:386–397.

Quade, J., et al. 2008. Paleowetlands and regional climate change in the central Atacama Desert, northern Chile. Quaternary Research 69:343–360.

Rasmussen, C., Matsuyama, N., Dahlgren, R. A., Southard, R. J., and N.Brauer.2007.

Soil genesis and mineral transformation across an environmental gradient on andesitic lahar. Soil Science Society of America Journal 71:225-237.

Rhoades, C., D. Binkley, H. Oskarsson, and R. Stottlemyer. 2008. Soil nitrogen accretion along a floodplain terrace chronosequence in northwest Alaska: Influence of the nitrogen-fixing shrub Shepherdia canadensis. Ecoscience 15:223–230.

Richardson, S. J., et al. 2004. Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. Oecologia 139:267–276.

Richardson, S. J., D. A. Peltzer, R. B.Allen, and M. S. McGlone. 2005. Resorption proficiency along a chronosequence: responses among communities and within species. Ecology 86:20-25.

Risacher, F., and B. Fritz. 1991. Quaternary geochemical evolution of the salars of Uyuni and Coipasa, central Altiplano, Bolivia. Chemical Geology 90:211-231.

Roche, M. A., Bourges, J., Cortes, J. and R. Mattos.1991. Climatologia e hidrologia de la cuencadel lago Titicaca. El Lago Titicaca, C. Dejoux and A. Iltis. ORSTOM. Paris. 83–104.

Rousk, J., Brookes, P. C., and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology75:1589-1596.

Rousk, J., et al. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4:1340-1351.

Sanz M. J., Carratalá A., Gimeno C., and M. M. Millán. 2002. Atmospheric nitrogen deposition on the east coast of Spain: relevance of dry deposition in semi-arid Mediterranean regions. Environmental Pollution 118:259-272.

Sarmiento, G. 2013. The dry plant formations of South America and their floristic

connections. Journal of Biogeography 2:233–251.

Schlesinger, W. H., and M. M. Hasey. 1981. Decomposition of chaparral shrub foliage: losses of organic and inorganic constituents from deciduous and evergreen leaves. Ecology 62:762-774.

Servicio Nacional de Geología y Minería (SGM) 1995. Carta geológica de Bolivia. Hoja Salinas de Garci Mendoza. Escala 1:250.000. Serie II-MTB-4B.

Servicio Nacional de Geología y Minería (SGM) 2002. Carta geológica de Bolivia. Hoja Villa Martin. Escala 1:250.000. Serie II-MTB-13B.

Servicio Nacional de Meteorología e Hidrología de Bolivia (Senamhi). 2011. Datos climáticos de las localidades de Uyuni, Salinas de Garci Mendoza y Llica. Potosi, Bolivia.

Sprent, J. I., and P. Sprent. 1990. Nitrogen Fixing Organisms. Chapman and Hall, London.

Steubing, L., and A. Fangmeier. 1992. Pflanzeno¨kologisches Praktikum. Berlin: Parey Verlag.

Taylor, B. R. 1998. Air-drying depresses rates of leaf litter decomposition. Soil Biology and Biochemistry 30:403–412.

Taylor, B. R., C. E. Prescott, W. J. F. Parsons, and D. Parkinson. 1991. Substrate control of litter decomposition in four Rocky Mountain coniferous forests. Canadian Journal of Botany 69:2242-2250

Tibaldi, A., C. Corazzato, and A. Rovida. 2009. Miocene–Quaternary structural evolution of the Uyuni–Atacama region, Andes of Chile and Bolivia. Tectonophysics 471:114–135.

Töwe, S., et al. 2010. Abundance of microbes involved in nitrogen transformation in the

rhizosphere of Leucanthemopsis alpina (L.) Heywood grown in soils from different sites of the Damma glacier forefield. Microbial Ecology 60:762-770.

Troll, C. 1968. Geo-ecology of the rnountain regions of the tropical Arnericas. Colloquiurn Geographicurn, vol. 9.Geographisches lnstitut der Universitat, Bonn.

Urcelay, C., Acho. J., and R. Joffre. 2011. Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation. Mycorrhiza 21:323–30.

Van Der Heijden, M. G., Bardgett, R. D., and N. M.Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296-310.

Vitousek, P. M., and H. Farrington. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37:63-75.

Vitousek, P. M., S. Hättenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and nature. AMBIO: A Journal of the Human Environment 31:97-101.

Vitousek, P. M., et al. 2002b. Towards an ecological understanding of biological nitrogen fixation Biogeochemistry 58:1–45.

Vitousek, P. M., Porder, S., Houlton, B. Z., and O. A. Chadwick. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20:5-15.

Vuille, M., Bradley, R. S., and F. Keimig. 2000. Interannual climate variability in the Central Andes and its relation to tropical Pacific and Atlantic forcing. Journal of Geophysical Research 105:12447-12460.

Walker, L. R., D. A. Wardle, R. D. Bardgett, and B. D. Clarkson. 2010. The use of chronosequences in studies of ecological succession and soil development Journal

of Ecology 98:725–736.

Walker, T. W., and J. K. Syers. 1976. The fate of phosphorus during pedogenesis. Geoderma 15:1-19.

Wardle, D.A., Hornberg, G., Zackrisson, O., Kalela-Brundin, M. and D. A. Coomes. 2003. Long-term effects of wildfire on ecosystem properties across an island area gradient. Science 300:972–975.

Wardle, D. A., Walker, L. R., and R. D. Bardgett. 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509-513.

Wardle, D. A., et al. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. Journal of Ecology 100:16-30.

Wright, I. J., and M. Westoby. 2003. Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. Functional Ecology 17:10-19.

Yahdjian, L., and O. E. Sala. 2010. Size of precipitation pulses controls nitrogen transformation and losses in an arid Patagonian ecosystem. Ecosystems 13:575-585.

Yahdjian, L., L. Gherardi, and O. E. Sala. 2011. Nitrogen limitation in arid-subhumid ecosystems : A meta-analysis of fertilization studies. Journal of Arid Environments 75:675–680.

Yavitt, J. B. 2000. Nutrient dynamics of soil derived from different parent material on Barro Colorado Island, Panama1. Biotropica32:198-207.

Zolá, R.P. and L. Bengtsson. 2007. Three methods for determining the area-depth relationship of Lake Poopó, a large shallow lake in Bolivia. Lakes and Reservoirs: Research and Management 12:275–284.

Table 1. Site characteristics across three long-term chronosequences from Andean Dry Puna, Bolivia.

Chronosequence	Parent material[£]	Formation time	Position	Elevation (m.a.s.l.)	Slope (%)	MAP[§] (mm)	MAT[§] (°C)
						8.5	176
Sedimentary	Undifferentiated sedimentary deposits	Pleistocene to Holocene	19°58 S, 68°11 W	3834	47.96 (19.09)		
Volcanic-1	Andesitic-basaltic domes	Pliocene to Holocene	19°46 S, 67°34 W	3878	48.01 (18.70)		
Volcanic-2	Dacitic domes	Pliocene to Holocene	20°21 S, 67°54 W	3866	33.46 (21.34)		

£= Data from SGM, 1995; 2002; Tibaldi et al, 2009.
§= MAT (Mean Annual Temperature) and MAP (Mean Annual Precipitation) from SENHAMI data base (1974-2008)

Table 2. Site characteristics across soil development on three chronosequences from Andean Dry Puna, Bolivia. Mean ± SE (Standard errors in parentheses) are given for each soil and plant characteristics.

Characteristics	Chronosequence			
	Sedimentary	Volcanic-1	Volcanic-2	*p*
Abiotic				
% C Total	**1.16** (0.17) ↓	0.91 (0.10)	1.31 (0.16)	0.07
% N Total	**0.03** (0.005) ↑	**0.06** (0.01) ↑	**0.08** (0.01) ↑	**
% P Total	**0.06** (0.002) ↓	0.07 (0.002)	0.07 (0.006)	*
P_{OLSEN} (mg kg^{-1})	12.86 (0.63)	18.33 (1.60)	**14.83** (0.85) ↓	**
C:N(molar)	**90.77** (26.16) ↓	24.33 (8.59)	**55.27** (34.96)	0.12
N:P (molar)	**1.21** (0.22) ↑	**1.90** (0.29) ↑	**2.55**(0.38) ↑	**
pH	**9.02** (0.08)	**8.33** (0.22) ↓	**7.93** (0.28) ↓	**
Soil moisture (gr H_2O/gr soil)	0.33 (0.01)	0.29 (0.01)	0.23 (0.01)	**
Salinity (dS m^{-1})	1.51 (0.30)	1.01 (0.16)	1.42 (0.32)	0.13
Biotic				
Plant cover (%)	**23.12** (2.15) ↓	**24.43** (1.49) ↓	**23.37** (2.10) ↓	0.69
A. imbricata cover (%)	**11.12** (1.44)	**8.81** (1.03)	**8.12** (1.19)	0.03
B. tola cover (%)	**4.93** (0.69)	4.50 (0.67)	**3.75** (0.63)	0.34
Leaf N (%)	**1.12** (0.07)	**1.21** (0.06)↑	1.28 (0.06)	*
Leaf P (%)	**0.10** (0.004)	**0.10** (0.006)	0.08 (0.005)	*

Values with arrows indicate statistical significant difference from one-way ANOVAs at $p < 0.05$ and trend (↑= increase; ↓=decline; and space = no tendency) across stages of ecosystem development for individual chronosequences.
p values indicate statistical significant difference from GLM Nested design ANOVA with stage nested in chronosequence (* $p < 0.05$; ** $p < 0.01$).

Table 3. Multiple regression analysis describing the effect of three predictor variables (selected by backward stepwise) on plant cover changes (response variable) across soil development.

Characteristics	Chronosequence					
	Sedimentary		Volcanic-1		Volcanic-2	
	Beta	p	Beta	p	Beta	p
Soil N:P	-1.23	<0.001	-	-	-	-
Soil pH	0.70	0.001	0.68	<0.001	0.66	<0.01
Soil salinity	-1.29	<0.001	-	-	-	-
R^2	**0.72**		**0.46**		**0.44**	
p	**0.001**		**<0.001**		**<0.001**	

Table 4. Multiple regression analysis describing the effect of three predictor variables (selected by backward stepwise) on belowground decomposition (response variable) across soil development.

Characteristics	Chronosequence					
	Sedimentary		Volcanic-1		Volcanic-2	
	Beta	p	Beta	p	Beta	p
Soil N:P	0.46	0.34	-	-	0.54	0.08
Soil pH	-0.45	0.14	-0.77	0.01	-0.57	0.01
Soil salinity	0.65	0.30	-	-	-	-
R^2	0.65		**0.60**		**0.51**	
p	0.19		**<0.01**		**0.02**	

Figure 1. Study area across Andean Dry Puna. **(a)**Map of Uyuni Salt Flat (Uyuni-SF) basin in west of South America, showing the surface covered by Tauca (dark gray) and Coipasa flood cycles (light gray). **(b)** Location of three types of chronosequences in the Uyuni Salt Flat. **(c)** Photography of Caracol chronosequence. Horizontal white lines shown level reached by flood events. **(d)** Graphical representation showing duration time (years) and elevation reached during flood events (mts).

Figure 2. Soil N and P patterns across soil development of three chronosequences. **(a)** Relationships between soil N:P and soil N showing the effect of soil N across stages and chronosequences driving changes in soil N:P. **(b)** Ordination of chronosequences relative to soil N and P, **(c)** Extreme N limitation among chronosequences. Dotted line indicates the N:P ratio above which soil is limited by P.

Figure 3. Soil and plant changes in three chronosequences from Andean Dry Puna **(a)** Changes in plant litter N:P across soil development, **(b)** Changes in plant cover (%) across soil development. **(c)** Changes in soil pH across soil development. **(d)** Changes in soil N:P across soil development.

Figure 4. Mean (± SE) of N concentration in leaf and litter in deciduous and evergreen species across soil development in sedimentary **(a, b)**, in volcanic-1 **(c, d)** and volcanic-2 chronosequences **(e, f)**. Dotted line indicates the threshold for complete resorption proficiency (*sensu* Killingbeck, 1996).

Figure 5. Mean (± SE) of N and P resorption efficiency in deciduous and evergreen species across soil development in sedimentary **(a, b)**, in volcanic-1 **(c, d)** and volcanic-2 chronosequences **(e, f)**.

Figure 6. Relationships between soil N:P and leaf and litter N:P across soil development in **(a,d)** sedimentary, **(b, e)** volcanic-1 and **(c, f)** volcanic-2 chronosequences. (white circle: leaf N:P and black circle: litter N:P)

Figure 7. Changes above and belowground decomposition across soil development. **(a)** Aboveground litter decomposition (expressed as percentage of weight loss) across soil development in sedimentary and volcanic-2 chronosequences (white square: sedimentary, and gray square: volcanic-2). Belowground decomposition across soil development in **(b)** sedimentary **(c)** volcanic-1 and **(d)** volcanic-2 chronosequences. (Different letter indicates significant differences among stages).

Figure S1. Mean (± SE) of P concentration in leaf and litter in deciduous and evergreen species across soil development in sedimentary **(a-b)**, in volcanic-1 **(c-d)** and volcanic-2 chronosequences **(e-f)**.

Figure 1.

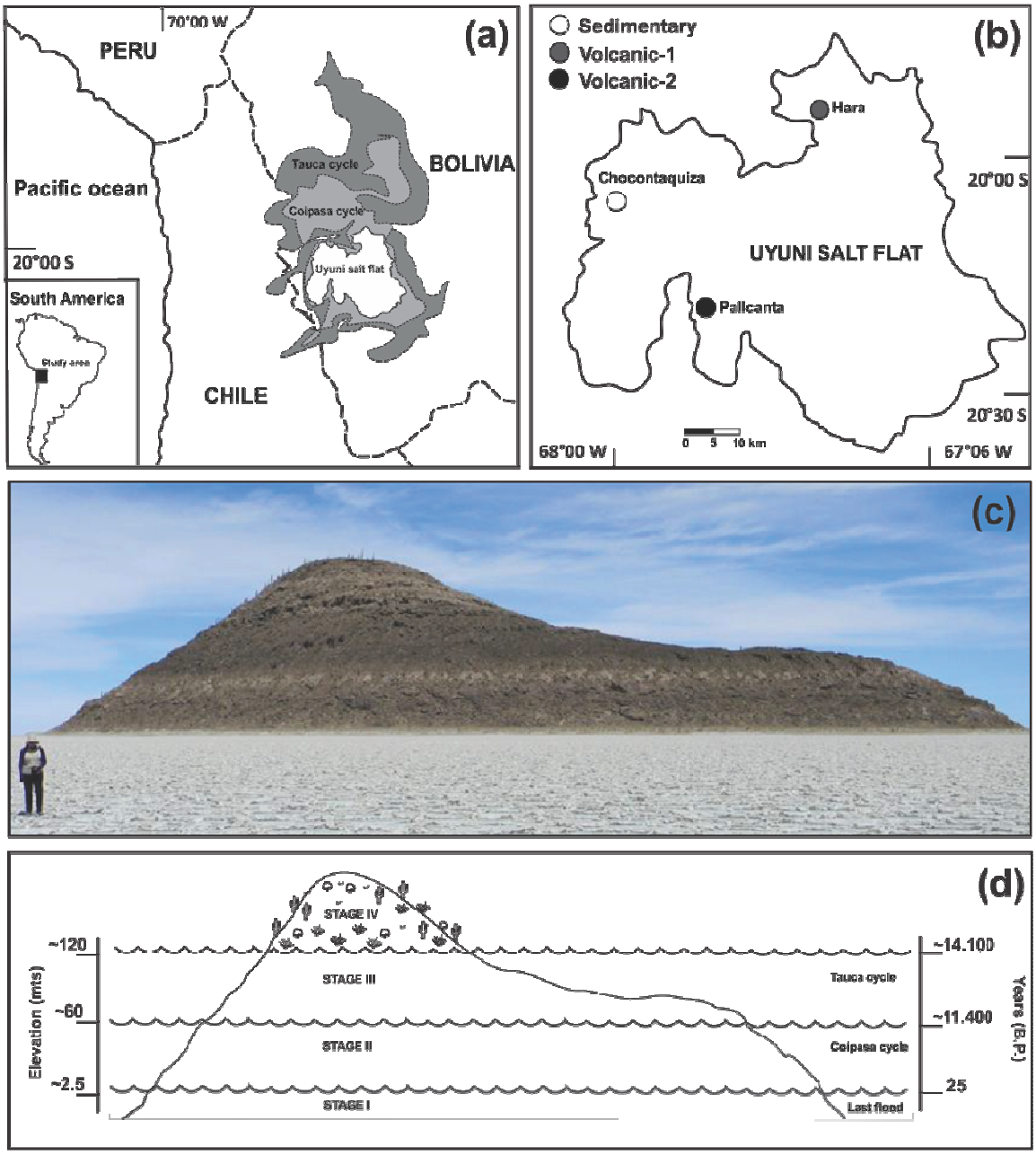

(a)
70°00 W
PERU
BOLIVIA
CHILE
Pacific ocean
20°00 S
Tauca cycle
Coipasa cycle
Uyuni salt flat
South America
Study area
(b)
Sedimentary
Volcanic-1
Volcanic-2
Hara
Chocontaquiza
20°00 S
UYUNI SALT FLAT
Pallcanta
20°30 S
68°00 W
0 5 10 km
67°06 W
(c)
(d)
STAGE IV
STAGE III
STAGE II
STAGE I
~120
~60
~2.5
Elevation (mts)
Tauca cycle
Coipasa cycle
Last flood
~14.100
~11.400
25
Years (B.P.)

Figure 2.

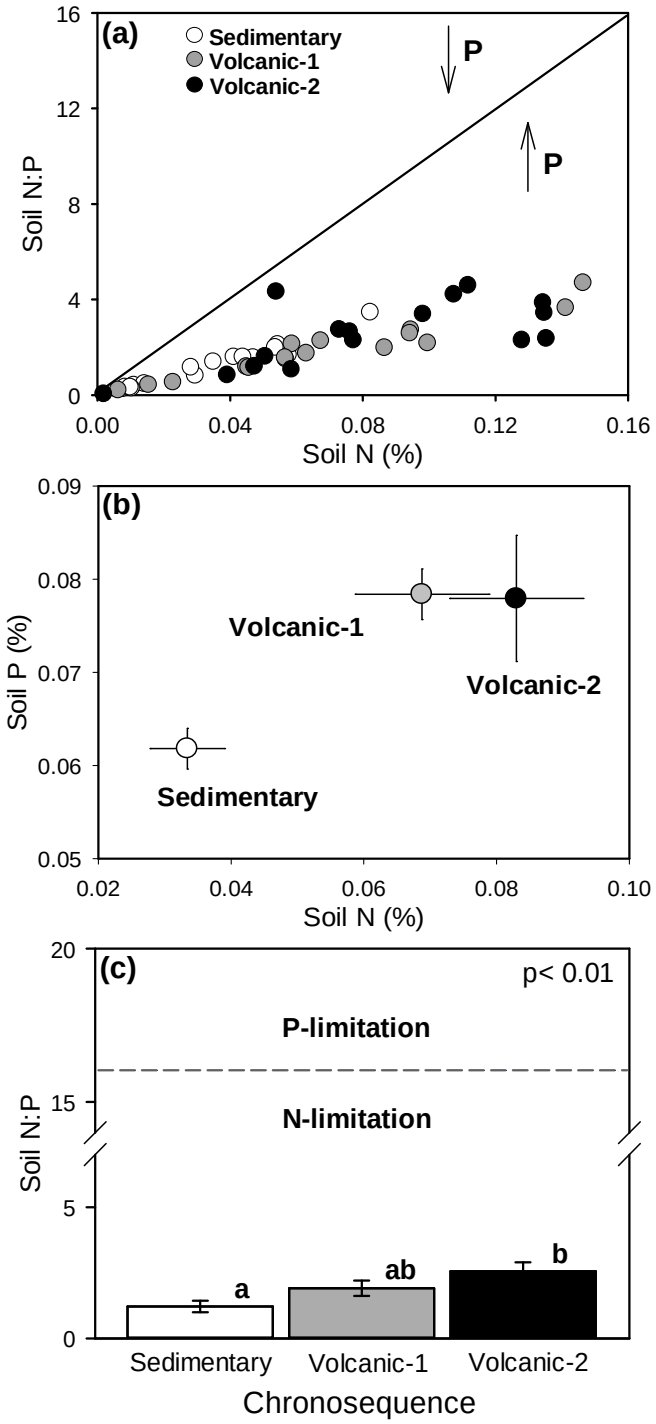

(a)
Sedimentary
Volcanic-1
Volcanic-2
P
P
Soil N:P
Soil N (%)
(b)
Volcanic-1
Volcanic-2
Sedimentary
Soil P (%)
Soil N (%)
(c)
p< 0.01
P-limitation
N-limitation
Soil N:P
a
ab
b
Sedimentary
Volcanic-1
Volcanic-2
Chronosequence

Figure 3.

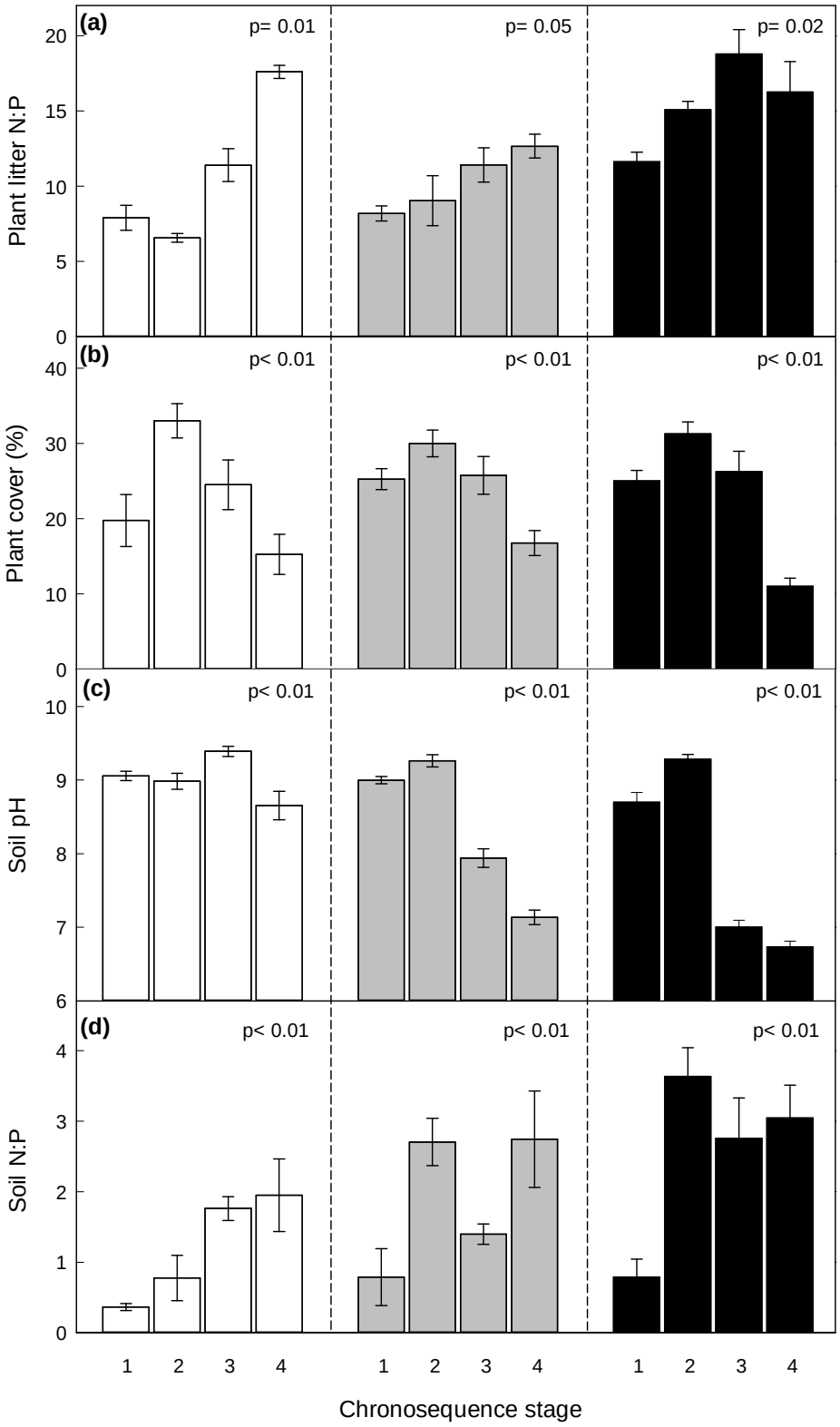

(a)
p= 0.01
p= 0.05
p= 0.02
Plant litter N:P
0
5
10
15
20
(b)
p< 0.01
p< 0.01
p< 0.01
Plant cover (%)
0
10
20
30
40
(c)
p< 0.01
p< 0.01
p< 0.01
Soil pH
6
7
8
9
10
(d)
p< 0.01
p< 0.01
p< 0.01
Soil N:P
0
1
2
3
4
1
2
3
4
1
2
3
4
Chronosequence stage

Figure 4.

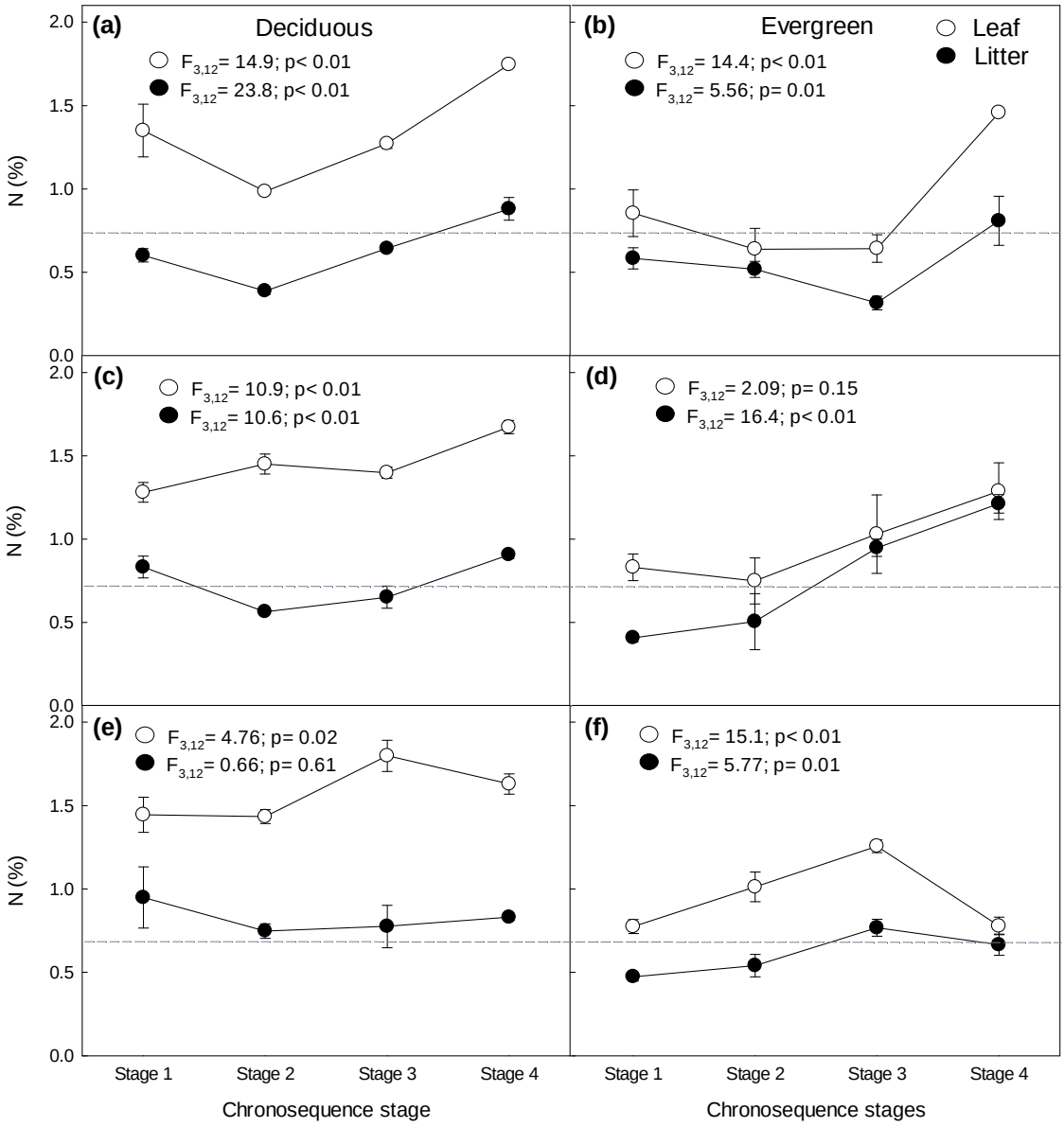

(a)
Deciduous
F3,12= 14.9; p< 0.01
F3,12= 23.8; p< 0.01
(b)
Evergreen
Leaf
Litter
F3,12= 14.4; p< 0.01
F3,12= 5.56; p= 0.01
(c)
F3,12= 10.9; p< 0.01
F3,12= 10.6; p< 0.01
(d)
F3,12= 2.09; p= 0.15
F3,12= 16.4; p< 0.01
(e)
F3,12= 4.76; p= 0.02
F3,12= 0.66; p= 0.61
(f)
F3,12= 15.1; p< 0.01
F3,12= 5.77; p= 0.01
N (%)
0.0
0.5
1.0
1.5
2.0
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage
Chronosequence stages

Figure 5.

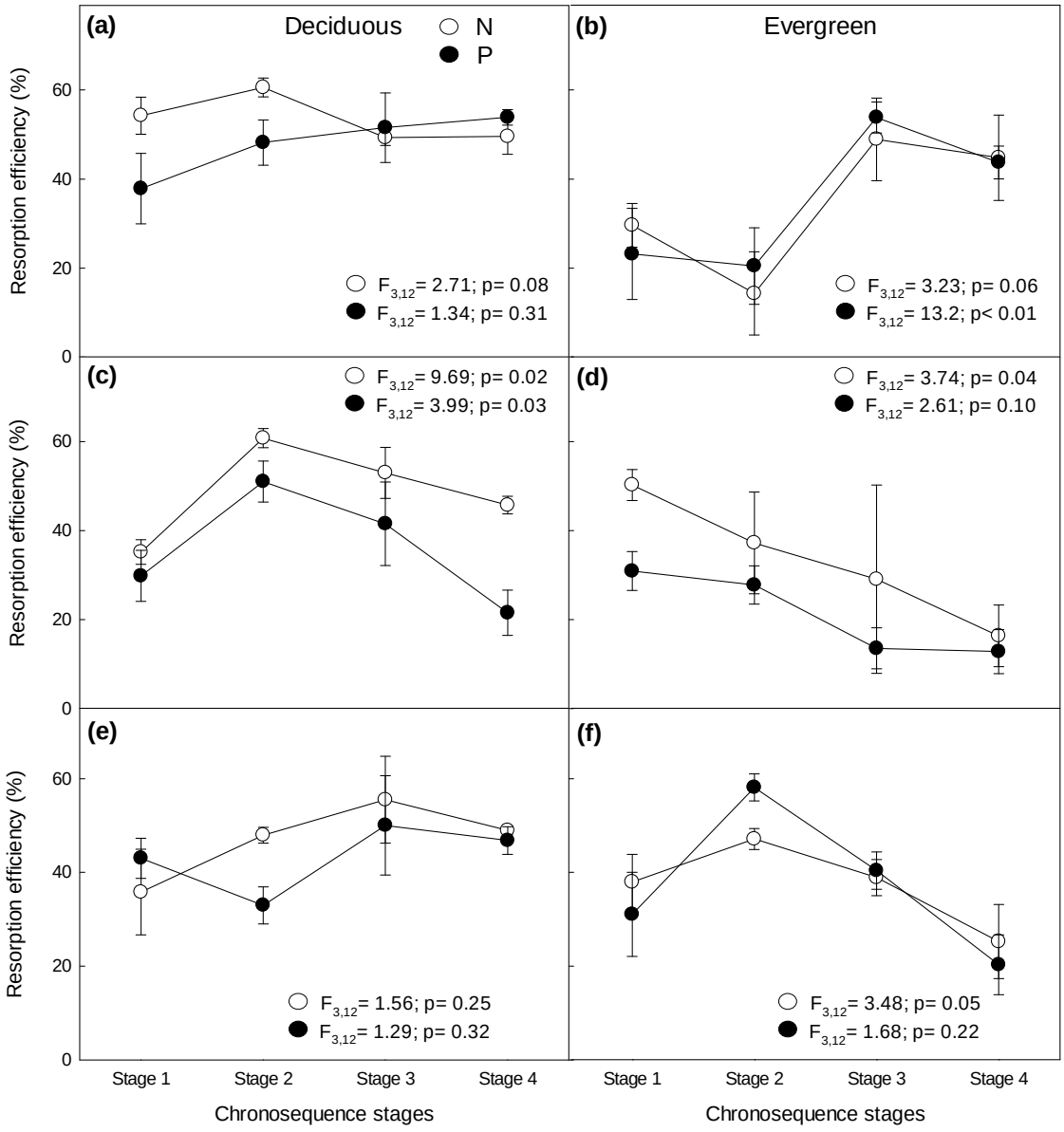

(a)
Deciduous
N
P
(b)
Evergreen
(c)
(d)
(e)
(f)
Resorption efficiency (%)
0
20
40
60
$F_{3,12}$= 2.71; p= 0.08
$F_{3,12}$= 1.34; p= 0.31
$F_{3,12}$= 3.23; p= 0.06
$F_{3,12}$= 13.2; p< 0.01
$F_{3,12}$= 9.69; p= 0.02
$F_{3,12}$= 3.99; p= 0.03
$F_{3,12}$= 3.74; p= 0.04
$F_{3,12}$= 2.61; p= 0.10
$F_{3,12}$= 1.56; p= 0.25
$F_{3,12}$= 1.29; p= 0.32
$F_{3,12}$= 3.48; p= 0.05
$F_{3,12}$= 1.68; p= 0.22
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stages

Figure 6.

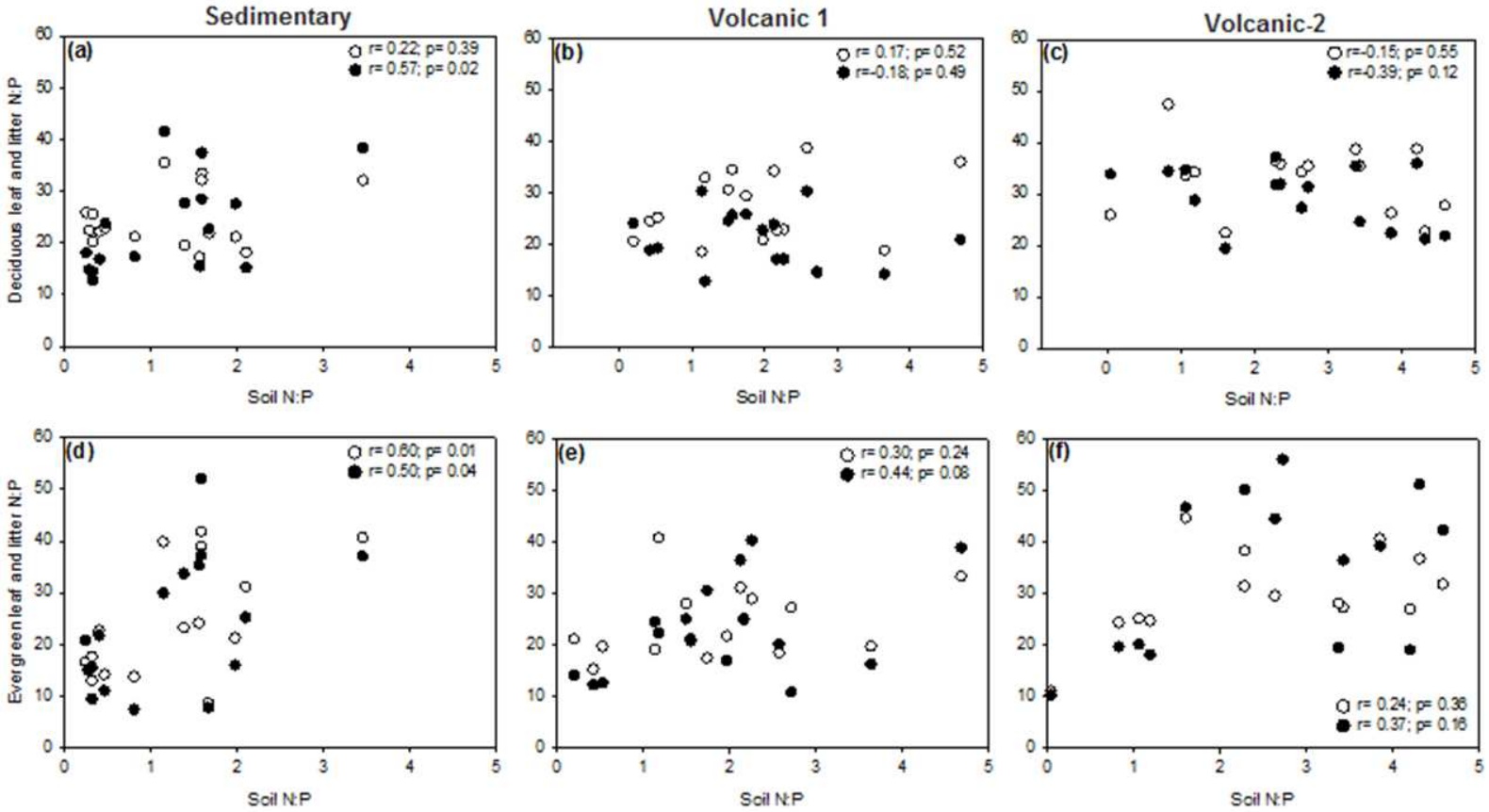

Sedimentary
Volcanic 1
Volcanic-2
(a)
r= 0.22; p= 0.39
r= 0.57; p= 0.02
(b)
r= 0.17; p= 0.52
r=-0.18; p= 0.49
(c)
r=-0.15; p= 0.55
r=-0.39; p= 0.12
(d)
r= 0.60; p= 0.01
r= 0.50; p= 0.04
(e)
r= 0.30; p= 0.24
r= 0.44; p= 0.08
(f)
r= 0.24; p= 0.36
r= 0.37; p= 0.16
Deciduous leaf and litter N:P
Evergreen leaf and litter N:P
Soil N:P

Figure 7.

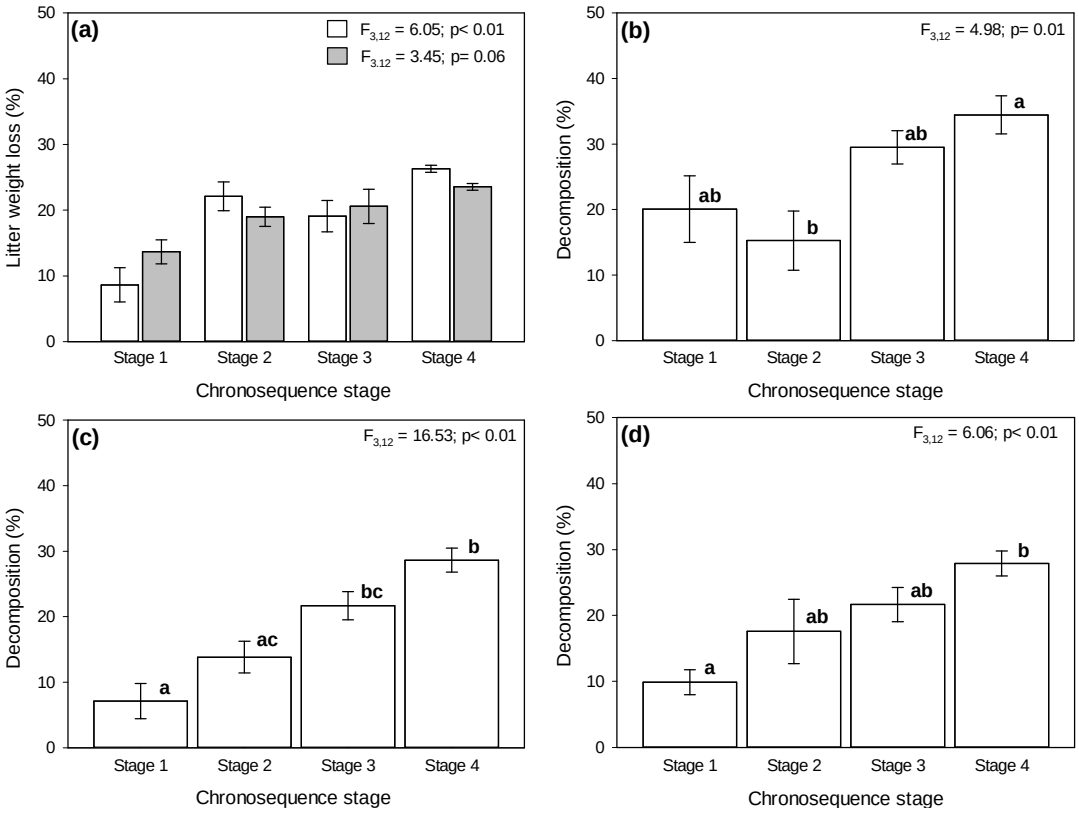
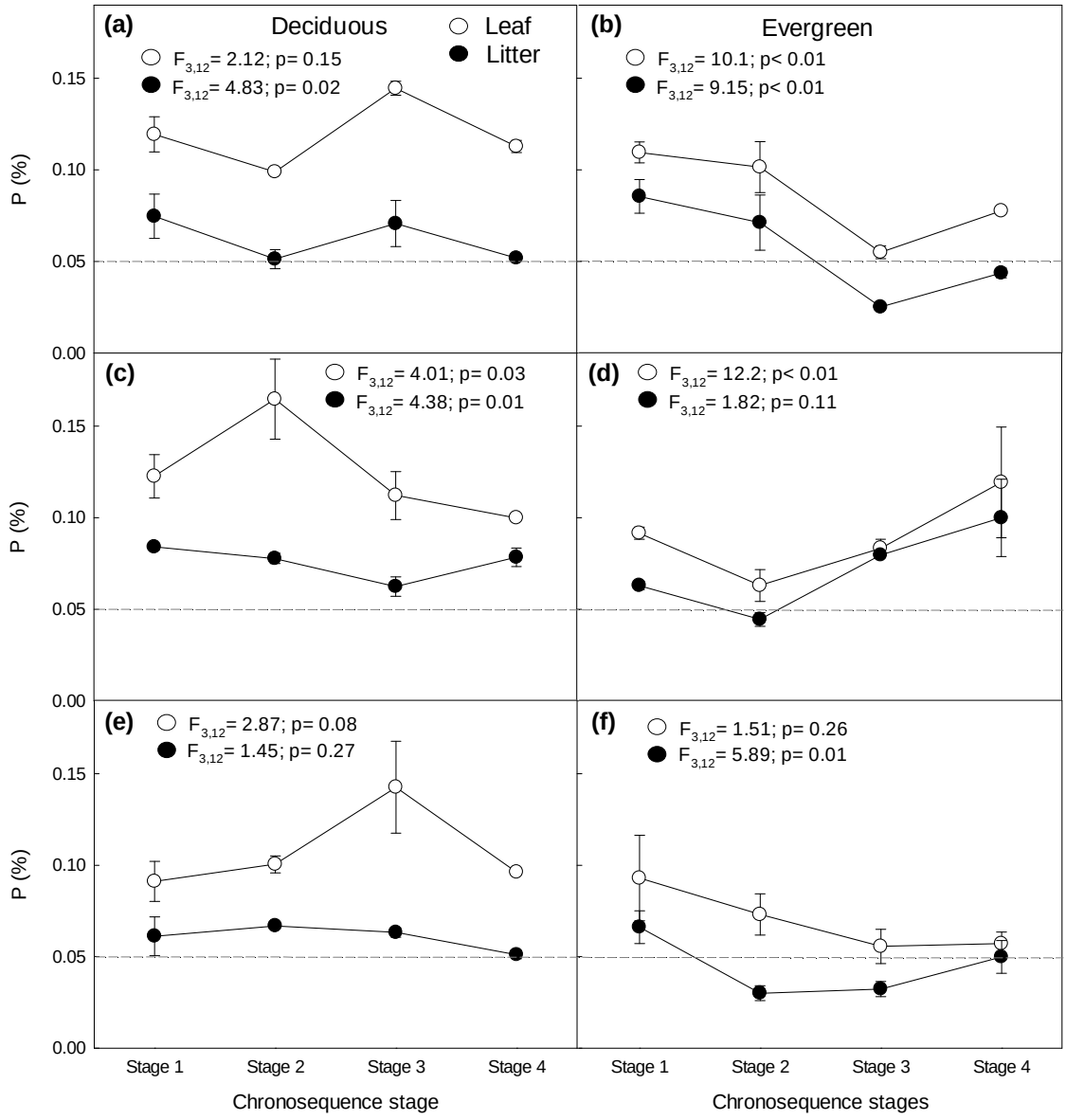

(a)
$F_{3,12}$ = 6.05; p< 0.01
$F_{3,12}$ = 3.45; p= 0.06
Litter weight loss (%)
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage
(b)
$F_{3,12}$ = 4.98; p= 0.01
Decomposition (%)
ab
b
ab
a
Chronosequence stage
(c)
$F_{3,12}$ = 16.53; p< 0.01
a
ac
bc
b
(d)
$F_{3,12}$ = 6.06; p< 0.01
a
ab
ab
b

Figure S1


(a)
Deciduous
Leaf
Litter
F3,12= 2.12; p= 0.15
F3,12= 4.83; p= 0.02
(b)
Evergreen
F3,12= 10.1; p< 0.01
F3,12= 9.15; p< 0.01
(c)
F3,12= 4.01; p= 0.03
F3,12= 4.38; p= 0.01
(d)
F3,12= 12.2; p< 0.01
F3,12= 1.82; p= 0.11
(e)
F3,12= 2.87; p= 0.08
F3,12= 1.45; p= 0.27
(f)
F3,12= 1.51; p= 0.26
F3,12= 5.89; p= 0.01
P (%)
0.00
0.05
0.10
0.15
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage
Chronosequence stages

CAPÍTULO II

What drives plant species richness across high Andean chronosequences?

ABSTRACT

Changes in plant species richness and composition during long-term soil development in mesic environments are frequently associated to shifts in abiotic factors that covariate with soil age such as soil Phosphorus availability. Biogeochemical gradients across soil development often represents soil stress gradients that could modified the importance relative of ecological interactions such as facilitation and competition, and affect thereby species richness patterns. Here, we evaluated the effect of changes in soil properties on plants species richness and composition during soil development in three primary successional c. 20,000-years chronosequences with soils derived from different parent material in the Andean Dry Puna, Bolivia. Further, we evaluated the effect of changes in plant-plant interactions on plant species richness during soil development. Our results indicate that plant species richness pattern during soil development were different among chronosequences, with increases in volcanic-1 and volcanic-2 chronosequences and declining in sedimentary chronosequence. Increases in soil N:P and declining in soil pH and salinity during soil development were the major determinants of plant species richness in volcanic-1 and volcanic-2 chronosequences. At difference of patterns observed in chronosequences from mesic environments, annual and perennial species showed similar pattern during soil development in all chronosequences. On the other hand and contrary to expectations facilitation declined during soil development and this pattern was similar in all chronosequences. As soil age increased and soil conditions improved competition shift to neutral plant-plant interactions. Overall, these results highlight the relative contribution of parent material and ecological interactions on plant species richness patterns during soil development, and reinforce the general observation that plant-plant interactions are the main driver of plant diversity patterns in arid ecosystems.

INTRODUCTION

Changes in plant communities along soil chronosequences are among the most conspicuous patterns in ecosystem ecology (Kitayama et al., 1995; Coomes et al., 2005; Vitousek et al., 2010; Wardle et al., 2012). Increases in species richness across soil-age gradients are commonly observed in long-term soil chronosequences from mesic subtropical to boreal zones (Richardson et al., 2004; Coomes et al., 2005; Wardle et al., 2008; Laliberté et al., 2103). The mechanism behind this general pattern among different plant forms (e.g. trees, shrubs and herbaceous species) is not clear (Wardle et al., 2004), however, abiotic factors that covariate with soil age such as Nitrogen (N) and Phosphorus (P) availabilities have been suggested as plausible explanations (Wardle et al., 2008; Carlson et al., 2010). For example Wardle et al, (2008) found that in six long-term chronosequences from different environments, limitation by total soil P during retrogressive stages was associated to increase in total vascular plants diversity.

Recent reviews comparing soil chronosequences from different environments such as boreal, subtropical mesic and template semiarid, suggest that climate could modulate soil development by alter the balance between inputs and losses of soil nutrients (Peltzer et al., 2010; Laliberte et al., 2013). Since climate interacts with the parent material to drive weathering and leaching from rock substrates during soil formation, the rates at which mineral nutrients are released to become available for plant assimilation will vary among soils developed under distinct climate conditions (Anderson, 1988; Huggett, 1998; Lichter, 1998; Esperschütz et al., 2011). For example, evidence from chronosequences in mesic boreal and temperate regions suggests that the P content of parent material and the amount of P remaining in weathered soils are major factors governing both, net primary production (Wardle et al., 2004) as well as plant composition and diversity variations across soil

development (Coomes et al., 2005; Wardle et al., 2008; Lambers et al., 2010). While, in semiarid environments gradual increasing of soil N and change in soil pH from alkaline young soils to neutral old soils drive soil development and plant productivity across long-term soil development (Zuo et al., 2011; Alfaro et al., 2013 Chapter I). However, how these biogeochemical gradients affect plant diversity and community composition across soil development in semiarid ecosystems never has been evaluated.

The nature of plant-plant interactions and their net effect on plant diversity is strongly dependent of resource supply (e.g. nutrient, water) and non-resource stress (e.g. salinity, pH) conditions (Bertness and Callaway, 1994; Pugnaire et al., 2004; Maestre et al., 2009). In turn, changes in soil structure and nutrient availability across soil development could modulate the strength and direction of plant-plant interactions (Wardle et al., 2008; Laliberte et al., 2013). For example, Coomes et al, (2005) showed that gymnosperms were outcompeted by angiosperms from the most fertile and youngest site of a soil chronosequence in southern New Zealand, whereas gymnosperms dominated in old, P-poor and waterlogged sites. Therefore, in long-term chronosequences large soil biogeochemical gradients of resource availability (e.g. soil N and P) and abiotic stress (e.g. soil pH) could affect the structure and composition of plant communities by filtering species along different phases or by modifying the strength and direction of plant interactions (Coomes et al., 2005; Gaxiola et al., 2010; Peltzer et al., 2010; Selmants and Hart, 2010).

Positive interactions are a strong force behind community structure and diversity patterns in semiarid ecosystems (Pugnaire et al., 1996; Schlesinger et al., 1996; Maestre et al., 2003). Water and nutrient limitation and environmental stress drive positive interactions, increasing cooperation as stressful conditions increase in these environments (Maestre and Cortina, 2004; Pugnaire et al., 2004; Armas et al., 2011). As long-term soil

development proceeds, primary productivity changes from early poor plant communities to intermediate more productive communities (Wardle et al., 2004). This increase in productivity is tightly associated with increase in soil N-availability through the time (Kitayama, 1996; Peltzer et al., 2010). Therefore, as soil development proceeds and productivity increases, plant-plant interactions are expected to change from positive to negative as has been previously suggested (Grime, 1973; Wilson and Tilman, 2002). However, these links between biogeochemical changes during soil development and ecological interactions has never been evaluated in semiarid ecosystems, and it is far from clear whether changes in the balance between facilitation and competition during soil development underlie plant species diversity patterns in soil chronosequences.

Based on these considerations we conducted our study along a soil chronosequence system located in the Central Andean Dry Puna, Bolivia (see Alfaro et al., Chapter I) to evaluate the relationships between soil biogeochemical changes and plant-plant interactions along soil age gradient; beside their effects on plant diversity and community composition patterns during long-term soil development. Specifically, we hypothesized that: (i) increases in soil nutrient availability (notably N) would promote higher species richness across soil development. (ii) annual and perennial plants will show different patterns during soil development, as these two groups represent different strategies for nutrient-use and conservation. (iii) increases in soil N and declines in soil salinity (i.e. abiotic stress), as soil development proceeds, would determine that the frequency and importance of positive interactions decline during soil development. (iv) plant community composition will change along stages of soil development, with more abundance of annual plant species during late stages of development (where more resources are available), whereas shrubs and perennial grasses will dominate early stages where stressful soil conditions prevail.

METHODS

Chronosequence system

Based in our previous studies in the Uyuni-SF chronosequence system (Alfaro et al., Chapter I) we selected three chronosequences that share similar attributes such as history of disturbance, climate conditions, distance from continent, maximum elevation as well as slope, plant cover and composition, and only differ in area and geological origin of the substrate (Table 1). The study area is a cold semiarid plain, where mean annual precipitation is 176 ± mm and mean annual temperature is 8.5°C (data for last 40 years), with freezing temperatures ~200 days of year (data from Meteorology and Hydrology National Service, Bolivia). More details in Alfaro et al, (Chapter I)

Sample collection

Across four stages of soil development of each soil chronosequence soil and plant samples were collected in April-May from 16 plots (30 x 2 m) to assess the effect of soil biogeochemical gradients (e.g. soil nutrients) and soil salinity (e.g. stress factor) on plant species richness and plant interactions. On each plot three soil subsamples (each spaced by 20 m) were collected and then were pooled into a single sample. Physicochemical analyses of soil samples are detailed in Alfaro et al, (Chapter I). We recorded separately diversity of annual and perennial species because we intended to analyze the responses of different functional groups to environmental variation independently. Further, we recorded biomass and number of annual plants present beneath and outside the two dominant shrub species *Atriplex imbricata* and *Baccharis sp*. (henceforth referred to by genus only). We used quadrants of 0.5 m^2 to record abundance, richness and biomass of seedlings that were

identified to species level when possible, in different microhabitats: (i) in the understory of *Atriplex* (ii) in the understory of *Baccharis* and (iii) in gaps between shrubs.

Data analysis

Plant sampling efficiency was evaluated by individual-based rarefaction curves (EcoSim 7.0, Gotelli and Entsminger, 2001). Within each chronosequence we used partial least squares regressions (PLSRs) to determine the extent to which soil factors predicted species richness (mean spp/plot) of annual and perennial plant species across soil development in the three chronosequences. We followed Carrascal et al, (2009) in reporting the relative contribution of first four soil factors that had major effects on plant species richness across soil development, as the square of predictor weights in the first component of PLSR. When variance explained in the response variable of the second component was < 5% only the first component was considered to express the results (Carrascal et al., 2009). To examine differences in species richness among stages of soil development we performed a one way analysis of variance (ANOVA). Significance levels were set at $\alpha = 0.05$ for all ANOVAs, for post-hoc comparisons between stages were performed using Tukey's HSD test. All data were tested for homogeneity of variances and standardized if necessary to meet ANOVA assumptions. All mean values are shown with ±1SE. Changes in plant community composition across soil development was evaluated by Redundancy Analysis (RDA) (Legendre and Gallagher, 2001) using Bray-Curtis dissimilarity matrices and one-way PERMANOVA. The relationship between variability in soil factors and changes in plant community composition across soil development was evaluated by the correlation between the first axis of RDA ordination of plant composition and soil values.

To assess the intensity, importance and type of plant-plant interactions (i.e. competition or facilitation) we used two indices; the Relative Interaction Index (RII, Armas et al., 2004) and the Interaction Importance Index (I_{IMP} Brooker et al., 2005). These indexes range from pure competition (-1) to pure facilitation (+1) interactions. RII = (P+N) – (P-N)/(P-N) + (P+N); where P represents the performance of beneficiary species in the presence (+N) or absence (-N) of shrubs. I_{IMP} = (P+N - P-N)/ (P+N - P-N) + (P-N – MB); where MB is the maximum value of the community richness on the plots. We calculated the RII and I_{IMP} indexes at community level by pooling individual values of all species within each stage of chronosequence. Multiple regression analyses were used to assess the effect of soil factors on RII and I_{IMP} index across oil development on each chronosequence. Because of different effect of resource supply (e.g. nutrient, water) and non-resource stress (e.g. salinity, pH) on plant-plant interactions, both factors were analyzed separately. Statistical analyses were performed using Statistica 9.0 (StatSoft Inc., Tulsa, Oklahoma, USA) and R ver. 2.9.1 (Development Core Team 2009).

RESULTS

Soil development

As expected soil N and N:P increased across soil development in all chronosequences. However, large values of N:P during late stages of development were determined by increases in N and not by decreases in soil P. Soil pH, salinity and moisture declined during soil development in all chronosequences; however, soil moisture was not significantly different across stages of soil development in all chronosequences (Table 1). All these findings suggest that across soil development there is a gradient of increasing N supply

from early to late stages of development, and a stress gradient from early saline and alkaline soils to late stages with less harsh conditions.

Variation in plant species richness across soil development

We found 52 plant species (22 perennial and 30 annual) in 48 plots across the three chronosequences. The accumulated species richness was 37 on each chronosequence (Table 2). The overall mean species richness per plot was 19 (9 perennial and 10 annual) and was significantly different among chronosequences ($F_{2,45}$= 65.27; p< 0.01), with volcanic-2 and sedimentary chronosequences showing the highest and lowest values respectively (Table 2). Across soil development plant species richness (spp/plot) increased significantly in the volcanic-1 and volcanic-2 chronosequences (Fig. 2a), while in the sedimentary chronosequence plant species richness declined with soil age although not significantly (Fig. 2b). When these patterns were analyzed by plant life form, annual species richness increased significantly with soil age in the volcanic-2 chronosequence ($F_{3,12}$= 8.39; p= 0.01) and declined in the sedimentary chronosequence ($F_{3,12}$= 5.07; p= 0.01); while perennial species richness only increased during soil development in the volcanic-2 chronosequence ($F_{3,12}$= 7.03; p= 0.01).

Partial least squares regression (PLSRs) analysis permitted to select the combination of soil factors that account for the major proportion of the variance in plant species richness patterns during soil development. Across the first component of PLSR, soil P_{OLSEN}, N:P, pH and salinity were the soil factors that significantly predicted changes in species richness during pedogenesis. Coefficient of PLSRs analyzes indicated that these four soil factors grouped in the first component were significant predictors of variation in plant species richness in volcanic-1 (R^2= 0.61; p= 0.001) and volcanic-2 chronosequences

(R^2= 0.48; p= 0.001) (Table 3). In contrast, in the sedimentary chronosequence plant richness changes were not significantly correlated with any set of predictor variables (R^2= 0.16; p= 0.09; Table 3). The relative contribution of soil factors on plant species richness patterns during pedogenesis was largely different between volcanic-1 and volcanic-2 chronosequences. Accordingly, in the volcanic-1 chronosequence soil salinity and soil pH explained around of 66 % of variation in plant species richness. Meanwhile in volcanic-2 chronosequence soil P_{OLSEN} and soil N:P accounted of 66 % of variance in plant species richness across soil development (Table 3). Clearly species richness increases in the volcanic-1 chronosequence was affected by nutrient pool changes, whereas in the volcanic-2 chronosequence this pattern was determined by changes in soil stressors (i.e. soil pH and salinity) (Table 3).

Separate analyses for plant life forms indicated that relative contribution of soil factors was largely different for annual and perennial species richness patterns during soil development. The first component of PLSR explained different portion of variation for annual and perennial species richness during soil development on each chronosequence. For example, in the sedimentary chronosequence declines in species richness of annual plants was mostly determined by decreases in soil N:P and declines in soil salinity (Fig. 3a). Richness of perennial species was mainly determined by changes in soil P_{OLSEN} and soil salinity (Fig. 3b). In the volcanic-1 chronosequence the first component of PLSR explained 58% and 48% of variation in annual and perennial plant species richness increase respectively, in both cases declines in soil salinity and pH were the main determinants (Fig. 3a, 3b). In volcanic-2 chronosequences the first component of PLSR explained 18% and 59% of variation in annual and perennial species richness, respectively, where declines in soil pH and P_{OLSEN} driving increases of annual species richness (Fig. 3a); while soil N:P

and P_{OLSEN} changes determined increases in perennial species richness during soil development.

Changes in plant community composition across soil development

One-way PERMANOVA results from first axis of RDA ordination indicated that annual and perennial plant communities changed across soil development in all types of chronosequences (Fig. S1). Of all species recorded 43%, 65% and 57 % occurred in more than two stages across soil development in sedimentary, volcanic-1 and volcanic-2 chronosequences respectively. Plant communities from youngest stages of development were significantly distinct from communities in late stages in all chronosequences (Fig. S1).

Correlation between the first axis of RDA ordination of annual and perennial community composition and soil properties showed that shifts in soil nutrient properties and changes in plant richness and cover drive plant species turnover across soil development, in particular, increases in soil N:P, decline in soil pH, salinity and soil moisture were associated to changes in plant composition across soil development in all chronosequences (Table 5). These results indicate that despite the short distance among stages of development, increases in soil N:P along soil age gradient was a key driver of shifts in annual and perennial community composition in all chronosequences. Contrary to our expectations relative abundance (corrected by cover) of annual plants declined during late stages of development in sedimentary and volcanic-2 chronosequences, meanwhile, abundance of perennial and cacti species remained constant across soil development in all three chronosequences (Fig. S2).

Variation in plant-plant interactions across soil development

At three chronosequences higher species richness and biomass were found in open areas compared to sites below *Atriplex* and *Baccharis* (Table S1). We found that in contrast to open areas, richness of annual species declined below shrubs during early stages, shifting to equal richness below shrubs and open areas during late stages of development in all chronosequences (Fig. 4). Contrary to our expectations, increases in annual species beneath shrubs was positively related to increases in soil N:P across soil development in sedimentary and volcanic-1 chronosequences (Table 4), suggesting that in these two chronosequences soil resources are more important than soil non-resource stress modulating plant-plant interactions. While in volcanic-2 chronosequence, in contrast, increases in annual species richness below shrubs across soil development were associated to decreases in soil pH (Table 4).

The effect of plant-plant interactions (RII) on annual species richness patterns across soil development was different among chronosequences. Since in sedimentary chronosequence species richness declined across pedogenesis, increases in RII of deciduous and evergreen and annual species richness were negatively related (R^2= -0.12; p= 0.17; R^2= -0.30; p= 0.02). Meanwhile, in volcanic-1 and volcanic-2 chronosequences increases in RII during soil development were positively related to increases in annual species richness (Fig 5b, 5c). In volcanic-2 chronosequence the contribution of deciduous and evergreen shrubs on annual species richness across soil development was similar (R^2= 0.36; p= 0.01; R^2= 0.39; p< 0.01).

DISCUSSION

Plant species richness across soil development

In contrast to previous comparative studies of plant richness patterns during soil development that includes soil chronosequences in retrogressive succession with low soil P in old stages (see Wardle et al., 2004), biogeochemical gradients and ecosystem processes strongly suggest that soil development in three chronosequences from Uyuni-SF are in the progressive phase (*sensu* Wardle et al., 2003), where soil N generally is the most limiting nutrient (Wardle et al., 2003; Richardson et el., 2004; Vitousek, 2004). Surprisingly, after ca. 20,000 years of soil development in the Uyuni-SF, soil N is largely the scarcest soil macronutrient, whereas high total P remained without significant variation across soil development independently of the geological origin of the chronosequence. Clearly in this Andean dry ecosystem soil development followed different patterns than reported previously across similar span of time in chronosequences from mesic environments (Wardle et al., 2004; 2008; Peltzer et al., 2010), which in turn affected plant diversity and composition during soil development.

In retrogressive chronosequences plant species richness increase monotonically during soil development and has been suggested that these patterns are related to change in soil nutrient variability (notably total P) (Laliberte et al., 2013). Large decline in total soil P during late stages of development would promote species diversity by increasing nutrient limitation stress, and thereby will reduce competitive exclusion (Richardson et al., 2004; Wardle et al., 2008). Our results provide partial support for these observations, because plant species richness increases during soil development in two of three chronosequences and was associated to changes in soil N:P and soil P availability (P_{OLSEN}). Because P_{OLSEN} is a measure of plant-available P has been suggested that its changes could drive plant

diversity and productivity during soil development (Partfitt et al., 2005). Indeed, the effect of soil P_{OLSEN} in volcanic-1 and volcanic-2 chronosequences could be showing that different forms of soil P could drive plant species richness patterns during soil development (Turner et al., 2007; Selmants and Hart, 2010; Holdaway et al., 2011).

Since different strategies to use and conserve resources, associated to different life strategies such as growth rate and development, have been suggested that distinct factors drive herbaceous and perennial plant diversity during soil development (Richardson et al., 2004; Peltzer et al., 2010). For example the first study that compared plant richness patterns across soil development in long-term chronosequences from subtropical to boreal zones (Wardle et al., 2008) showed that total vascular plants reached the maximal species richness in older stages with low productivity. Instead tree species richness declined during late successional stages and this pattern was related to large decline in total P during pedogenesis. In the Uyuni-SF perennial shrubs are the dominant woody species (no trees were recorded across the chronosequence system). At difference of Wardle et al, (2008) perennial species increased during soil development in volcanic-1 and volcanic-2 chronosequences in the Uyuni-SF. These different patterns respect to chronosequences from mesic environments (Richardson et al., 2004; Wardle et al., 2008) are determined by different trajectory of soil N and P during soil development. Meanwhile in volcanic-1 and volcanic-2 chronosequences in the Uyuni-SF soil N increased across soil development, in mesic chronosequences soil P limitation drives the declining in tree species richness during late stages of development (Wardle et al., 2008). Positive relationships between annual and perennial species richness during soil development in volcanic-1 and volcanic-2 chronosequences in the Uyuni-SF apparently were determined by large decline in perennial

cover during late stages of development that contributed to increase in annual species richness, in particular, shade-intolerant annual species.

Plant community composition across soil development

Our results provide strong evidence of a clear differentiation among plant communities on each stage of soil development, suggesting that both annual and perennial composition change in response to shifts in soil properties across soil development independently of type of chronosequence. More importantly, these patterns were strongly associated to increase in soil N:P. These observations are consistent with previous studies which highlight the effect of shift in soil N:P on plant composition across soil development (Richardson et al., 2004; Coomes et al., 2005; Wardle et al., 2008). A proof of this is that even the opposite patterns of plant species richness across soil development between sedimentary and the other two types of volcanic chronosequences, annual and perennial composition was similar among the three chronosequences (Table S2).

As soil age increase and soil properties change plant community composition and structure often change in predictable ways, with abundant fast growing species dominating during young and intermediate stages of development turning to be dominated by slow growing stress-tolerant species (*sensu* Grime, 1977) in older soils (Lambers et al., 2008; Mason et al., 2010). Across soil development in the Uyuni-SF large differences in plant composition among stages was not associated to change in resource-use strategies dominance. Indeed, the abundance (ind/plot) of different plant forms did not follow a clear pattern during soil development in all chronosequences. Instead, our results suggest that perennial evergreen species with typical stress tolerant strategies were dominant across soil development in all chronosequences. However, as soil conditions improve, the relative

abundance of species with higher demand of soil nutrients such as ferns (*Cheilantes sp*; *Notholaena nivea*) and some fast growing annual species (*Philibertia sp*; *Stipa curviseta*) increased during late stages of development. Indeed, fern species only were recorded after 10,000 years (second stage) of soil development in all chronosequences.

Soil development drive plant-plant interactions

While facilitation and competition often operate simultaneously in communities, it has been proposed that facilitation increases in importance relative to competition as resource supply declines (e.g. nutrient, water) and non-resource stresses increase (e.g. soil pH and salinity) (Bertness and Callaway, 1994; Maestre et al., 2009). In contrast to our expectations, and previous studies (Callaway et al., 2002; le Roux and McGeoch, 2010), our results showed that in early stages of soil development where soil conditions were harsher (e.g. low N:P and high salinity) relatively more species were found in open areas than below shrubs (deciduous or evergreen), suggesting an increase in negative plant-plant interactions in resource poor and stressful stages. Indeed, as soil age increases and soil conditions improve relatively more species were found below shrubs than in open areas, changing from negative in early stages to neutral or positive interaction frequency and importance during late stages of development. These findings are consistent with studies from water-limited environments where competition increased with environmental stress (Maestre and Cortina, 2004; Bowker et al., 2010; Soliveres et al., 2011).

We suggest that increase in facilitative interactions was determined by changes in soil pH and soil N during soil development. In early stages of development high soil pH and low soil N probably determined that soil harshness was higher for plant growth. During early stages of soil development generally soil pH is higher (~8-9) and decline as soil age

increase (Richardson et al, 2004), however in first stages in the USF chronosequences soil pH was higher (~9.2) and was declining during soil development. Certainly, the elevated levels of soil pH could reduce significantly the recruitment and growth of annual plants (Partel, 2002; Shuster and Diekmann, 2003), in particular because soils in Andean Puna are neutral (Salm, 1983). Soil N was low in young stages of soil development in all chronosequences, and was the soil factor that accounts for a major proportion of the explained variance of RII in sedimentary and volcanic-1 chronosequences. Negative effects of N limitation on plant growth are largely known (Vitousek and Howarth, 1991) and are a widespread phenomenon in water-limited environments (Yahdjian et al., 2011). However, the extremely low levels recorded in the Uyuni-SF could increase the competition by soil N, leading to reduced species recruiting in early stages of development. Largely the increase in RII across soil development was accompanied with an increase in soil N and decline in soil pH. In this light, has been suggested that frequency and importance of interactions will decrease in stressful conditions (i.e early stage) because several environmental conditions could prevent development of plant species, even below of benefactor plants (Maestre et al., 2009; Soliveres et al., 2011).

Ecological interactions and plant species richness patterns

While Andean shrubs have been previously reported to show larger positive effects on community structure and composition of herbaceous species (Lopez et al., 2009), in the Uyuni-SF the effect of dominant shrubs on annual plant community structure was largely negative during early stages of development and shifted across soil development. However, an increase in annual species below shrubs, during soil development, was positively associated to increases in annual plant species richness in volcanic-1 and volcanic-2

chronosequences. In other words, plant species richness was higher when shrubs and annual plant interaction was neutral or positive. Positive relations between species richness and facilitative interactions have been observed across aridity gradients (van Zonneveld et al., 2012) and grazing pressure (Howard et al, 2012). In relation to composition, annual plants recruiting below shrubs and open areas simultaneously were more frequently observed during early stages of development, while species occurring in open areas o below shrubs exclusively, were only recorded in late successional stages, suggesting that species with broader stress tolerance range are widespread in harsh early successional stages. In this light, probably successful species recruitment during late stages of soil development rely on particular set of abiotic conditions and soil resource heterogeneity which would lead to increase in plant species richness later in chronosequences.

Regardless the mechanisms involved, our results suggest that plant-plant interactions could affect the patterns of plant species richness across soil development by modifying the occurrence and successful recruitment of annual plant species during late successional stages. Our results claim to include ecological interactions into potential drivers of plant species richness patterns across long-term soil development.

ACKNOWLEDGEMENTS

We thank to Paola Villarroel, Daniela Rivera, Huber Villca, Alejandro Coca and Mauricio Torrico for helping with field and lab work. We are very grateful with Center of Biodiversity and Genetics (CBG) for logistic support for fieldwork. F.D. Alfaro was funded by a doctoral scholarship from CONICYT AT-24100099 and Russell E. Train Education for Nature Fellowships. This project was funded by ICM P05-002 and CONICYT PFB-023.

LITERATURE CITED

Alfaro, F. D., Gaxiola. A., P. A. Marquet. Hyper slow soil development after catastrophic disturbance: soil N-limitation is the source of eternal youth in long-term successions in the Central Dry Andes. *In prep.*

Anderson, D. W. 1988. The effect of parent material and soil development on nutrient cycling in temperate ecosystems. Biogeochemistry 5:71–97.

Armas, C., et al. 2004. Measuring plant interactions: a new comparative index.Ecology 85: 2682–2686.

Armas, C., Rodríguez-Echeverría, S., and F. I. Pugnaire. 2011. A field test of the stress-gradient hypothesis along an aridity gradient. Journal of Vegetation Science 22:818–827.

Bertness, M. D. and R. Callaway. 1994. Positive interactions in communities. Trends in Ecology & Evolution 9:191–3.

Bowker, M. A. et al. 2010. Competition increases with abiotic stress and regulates the diversity of biological soil crusts. Journal of Ecology 98:551–560.

Brooker, R.W., et al. 2005.The importance of importance. Oikos 109:63–70.

Carlson, M. L. et al. 2010. Community development along a proglacial chronosequence: are above-ground and below-ground community structure controlled more by biotic than abiotic factors? Journal of Ecology 98:1084–1095.

Carrascal, L. M. et al. 2009. Partial least squares regression as an alternative to current regression methods used in ecology. Oikos 118:681–690.

Callaway, R.M., et al. 2002. Positive interactions among alpine plants increase with stress: a global experiment. Nature 417:844–848.

Cavieres, L. et al. 2006. Positive interactions between alpine plant species and the nurse

cushion plant *Laretia acaulis* do not increase with elevation in the Andes of central Chile. The New Phytologist 169:59–69.

Coomes, D. A., et al. 2005. The hare, the tortoise and the crocodile: the ecology of angiosperm dominance, conifer persistence and fern filtering. Journal of Ecology 93:918–935.

Esperschütz, J., A. et al. 2011. Microbial food web dynamics along a soil chronosequence of a glacier forefield. Biogeosciences 8:1275–1308.

Gaxiola, A., McNeill, S. M., and D. A. Coomes. 2010. What drives retrogressive succession? Plant strategies to tolerate infertile and poorly drained soils. Functional Ecology 24:714–722.

Gotelli, N. J. and G. L. Entsminger. 2001. EcoSim: Null models software for ecology. Version 7.0.Acquired Intelligence Inc. & Keseyear.

Grace, J. B. 2001. The roles of community biomass and species pools in the regulation of plant diversity. Oikos 92:193–207.

Grime, J. P. 1973.Competitive exclusion in herbaceous vegetation. Nature 242:344–347.

Grime, J. P. 2001. Plant strategies, vegetation processes and ecosystem processes. Wiley.

Holdaway, R. J. et al. 2011. Species- and community-level patterns in fine root traits along a 120,000-year soil chronosequence in temperate rain forest. Journal of Ecology 99:954–963.

Howard, K. S. C. et al. 2012. Positive effects of shrubs on plant species diversity do not change along a gradient in grazing pressure in an arid shrubland. Basic and Applied Ecology 13:159–168.

Huggett, R. 1998. Soil chronosequences, soil development, and soil evolution: a critical review. Catena 32:155–172.

Kitayama, K., Mueller-Dombois, D., and P. M. Vitousek 1995. Primary succession of Hawaiian montane rain forest on a chronosequence of eight lava flows. Journal of Vegetation Science 6:211–222.

Kitayama, K. 1996. Soil nitrogen dynamics along a gradient of long-term soil development in a Hawaiian wet montane rainforest. Plant and Soil 183:253–262.

Laliberté, E. et al. 2012. Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. Journal of Ecology 100:631–642.

Laliberté, E. et al. 2013. How does pedogenesis drive plant diversity? Trends in Ecology & Evolution 28:331–340.

Lambers, H. et al. 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology & Evolution 23:95–103.

Lambers, H., Brundrett, M. C., Raven, J. A., and S. D. Hopper. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant and Soil 334:11–31.

Legendre, P., and E. Gallagher . 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.

Le Roux, P. C., and M. A. McGeoch. 2010. Interaction intensity and importance along two stress gradients: adding shape to the stress-gradient hypothesis. Oecologia 162:733–745.

Lichter, J. 1998. Rates of weathering and chemical depletion in soils across a chronosequence of Lake Michigan sand dunes. Geoderma 85:255–282.

Lopez, R. L, et al. 2009. Positive effects of shrubs on herbaceous species richness across several spatial scales: evidence from the semiarid Andean subtropics. Journal of

Vegetation Science 20:728–734.

Maestre, F. T., Bautista, S., and J. Cortina. 2003. Positive, negative, and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. Ecology 84:3186–3197.

Maestre, F. T., and J. Cortina. 2004. Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. Proceedings of the Royal Society, Biological Sciences 271:331–333.

Maestre, F. T. et al. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97:199–205.

Mason, N. W. H. et al. 2012. Changes in coexistence mechanisms along a long-term soil chronosequence revealed by functional trait diversity. Journal of Ecology 100: 678–689.

Michalet, R. 2006. Is facilitation in arid environments the result of direct or complex interactions? New Phytologist 169:3–6.

Peltzer, D.A., et al. 2010. Understanding ecosystem retrogression. Ecological Monographs 80:509–529.

Placzek, C. J., Quade, J., and P. J. Patchett. 2013. A 130ka reconstruction of rainfall on the Bolivian Altiplano. Earth and Planetary Science Letters 363:97–108.

Pugnaire, F. I., Haase, P., and J. Puigdefabregas. 1996. Facilitation between higher plant species in a semiarid environment. Ecology 77:1420–1426.

Pugnaire, F. I. and M. T. Luque. 2001. Changes in plant interactions along a gradient of environmental stress. Oikos 93:42–49.

Pugnaire, F. I., Armas, C., and F. Valladares. 2004. Soil as a mediator in plant-plant interactions in a semi-arid community. Journal of Vegetation Science 15:85–92.

Schlesinger, W. H., et al. 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77:364–374.

Selmants, P. C., and S. C. Hart. 2010. Phosphorus and soil development: does the Walker and Syers model apply to semiarid ecosystems? Ecology 91:474–84.

Servicio Nacional de Meteorología e Hidrología de Bolivia (Senamhi). Climatic stations from de Uyuni, Llica and Salinas de Garci Mendoza.

Soliveres, S. et al. 2011. Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: Towards a unifying framework. Perspectives in Plant Ecology, Evolution and Systematics 13: 247–258.

Turner, B. L. et al. 2007. Soil organic Phosphorus transformations during pedogenesis. Ecosystems 10:1166–1181.

Urcelay, C., Acho, J., and R. Joffre. 2011. Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation. Mycorrhiza 21:323–330.

Van Zonneveld, M. J. et al. 2012. Shrub facilitation increases plant diversity along an arid scrubland-temperate rain forest boundary in South America (R Halvorsen, Ed.). Journal of Vegetation Science 23:541–551.

Vitousek, P. M. and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry13:87–115.

Vitousek, P. M. 2004. Nutrient cycling and limitation.Hawaii as a model system. Princeton Univ. Press.

Vitousek, P. M. et al. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20:5–15.

Wardle, D.A., Hornberg, G., Zackrisson, O., Kalela-Brundin, M. and D. A. Coomes. 2003. Long-term effects of wildfire on ecosystem properties across an island area gradient. Science 300:972–975.

Wardle, D. A. et al. 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513.

Wardle, D. A. et al. 2008. The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. Oikos 117:93–103.

Wardle, D. A. et al. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. Journal of Ecology 100:16–30.

Whittaker, R. H. 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecological Monographs 30:279–338.

Wilson, S. D., and D. Tilman. 2002. Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. Ecology 83:492–504.

Yahdjian, L. et al. 2011. Nitrogen limitation in arid-subhumid ecosystems: A meta-analysis of fertilization studies. Journal of Arid Environments 75:675–680.

Zuo, X. A. et al .2011. A positive correlation between plant diversity and productivity is indirectly caused by environmental factors driving spatial pattern of vegetation composition in semiarid sandy grassland. Biogeoscience 8:11795–11825.

Table 1. Site and abiotic characteristics across soil development in three chronosequences from Andean Dry Puna, Bolivia. Mean ± SE (Standard errors in parentheses) are given for each soil characteristics.

Characteristics	Chronosequence			
	Sedimentary	Volcanic-1	Volcanic-2	*p*-value
Parent material	Sedimentary deposits	Andesitic-basaltic domes	Andesitic lava flows	
Position	19°58 S; 68°11 W	19°46 S; 67°34 W	20°21 S; 67°54 W	
Area (ha)	445.39	187.96	197.81	
Elevation	3834	3878	3866	
Slope (%)	47.96 (19.09)	48.01 (18.70)	33.46 (21.34)	
N:P (molar)	**1.21** (0.22) ↑	**1.90** (0.29) ↑	**2.55** (0.33) ↑	**
P_{OLSEN}	12.86 (0.63)	18.33 (1.60)	**14.83** (0.85) ↓	**
Soil pH	**9.02** (0.08) ↓	**8.33** (0.22) ↓	**7.93** (0.28) ↓	**
Soil moisture	0.33 (0.01)	0.29 (0.01)	0.23 (0.01)	**
Salinity[1]	**1.51** (0.30) ↓	**1.01** (0.16) ↓	1.42 (0.32)	0.13

Values in bold indicate statistical significant difference from one-way ANOVAs across ecosystem development in chronosequences; and arrow shown the pattern of shift as soil age increase (↑= increase; ↓=decline; and space = no tendency).
p values indicate statistical significant difference from GLM Nested design ANOVA with stage nested in chronosequence (* $p < 0.05$; ** $p < 0.01$).
[1]= Electrical conductivity (dS m^{-1}).

Table 2. Plant community characteristics across soil development in three chronosequences from Andean Dry Puna, Bolivia. Mean ± SE (Standard errors in parentheses) are given for each plant characteristics.

Characteristics	Chronosequence			
	Sedimentary	Volcanic-1	Volcanic-2	*p*-value
Accumulated plant richness	37	37	37	
Plant richness (mean spp/plot)	15.32 (0.59)	**20.52** (0.90)↑	**20.62** (0.88) ↑	**
Annual richness (mean spp/plot)	**8.06** (0.34)↓	**11.31** (0.69) ↑	11.25 (0.54)	**
Perennial richness (mean spp/plot)	7.25 (0.50)	9.18 (0.44)	**9.37** (0.68) ↑	*
Plant cover (%)	**23.12** (2.15) ↓	**24.43** (1.49) ↓	**23.37** (2.10) ↓	0.69

Values in bold indicate statistical significant difference from one-way ANOVAs across ecosystem development in chronosequences; and arrow shown the pattern of shift as soil age increase (↑= increase; ↓=decline; and space = no tendency).
p values indicate statistical significant difference from GLM Nested design ANOVA with stage nested in chronosequence (* $p < 0.05$; ** $p < 0.01$).

Table 3. Coefficients of first component of Partial least squares regression (PLSR 1) model for plant richness (mean spp/plot) across soil development in three chronosequences in Andean Dry Puna, Bolivia.

Soil driver	Chronosequences		
	Sedimentary	Volcanic-1	Volcanic-2
	PLSR 1	PLSR 1	PLSR 1
Soil P_{OLSEN}	0.59	0.13	0.34
Soil N:P	0.34	0.11	0.32
Soil pH	0.05	0.24	0.16
Soil salinity	0.02	0.52	0.18
R^2	0.16	**0.61**	**0.48**
P	0.09	**0.001**	**0.001**

Table 4. Results of multiple regression analysis carried out with RII in deciduous and evergreen shrubs (response variable) and resource (soil N:P and soil moisture) and non-resource variables (soil salinity and soil pH) describing large soil variability across soil development in three chronosequences in Andean Dry Puna, Bolivia. (In bold the standardized multiple regression coefficient that was significant at $p < 0.05$).

Soil variable	Chronosequences					
	Sedimentary		Volcanic-1		Volcanic-2	
	Deciduous	Evergreen	Deciduous	Evergreen	Deciduous	Evergreen
Resource						
Soil N:P	0.76	**0.91**	**0.57**	0.54	0.24	0.38
Soil moisture	0.15	-0.08	-0.36	-0.21	0.25	0.26
Non-resource						
Soil salinity	0.40	0.49	-0.05	0.18	0.06	0.07
Soil pH	-0.20	-0.19	-0.16	-0.15	**-0.62**	**-0.51**
R^2	0.27	**0.55**	**0.56**	0.35	**0.38**	**0.26**
P	0.44	0.04	0.04	0.25	0.01	0.04

Table 5. Pearson's correlation coefficient between the first axis score of the RDA ordination of annual and perennial plant community composition and environmental variables across three types of long-term chronosequences. In brackets the proportion of variance explained by the first axis of RDA.

Factor	Chronosequence					
	Sedimentary		Volcanic-1		Volcanic-2	
	Annual (0.59)	Perennial (0.58)	Annual (0.56)	Perennial (0.82)	Annual (0.53)	Perennial (0.88)
P_{OLSEN}	-0.05	-0.01	0.41	-0.29	-0.33	**-0.73**
N:P (molar)	**0.82**	**0.76**	0.05	**0.54**	**0.54**	**0.78**
pH	0.02	0.01	**0.63**	-0.29	-0.02	-0.35
Soil moisture	-0.38	-0.46	-0.13	-0.45	**-0.61**	**-0.64**
Salinity	**-0.71**	**-0.70**	0.03	-0.36	-0.45	-0.48
Plant species richness	-0.26	-0.22	-0.03	**0.61**	**0.51**	**0.82**
Plant cover	-0.24	-0.06	**-0.80**	-0.09	-0.31	-0.17

Note: values in bold indicate statistical significance at $p < 0.05$.

Figure 1. Study area across Andean Dry Puna. **(a)** Map of Uyuni Salt Flat (Uyuni-SF) basin in west of South America, showing the surface covered by Tauca (dark gray) and Coipasa flood cycles (light gray). **(b)** Location of three types of chronosequences in the Uyuni Salt Flat. **(c)** Photography of Caracol chronosequence. Horizontal white lines shown level reached by flood events. **(d)** Graphical representation showing duration time (years) and elevation reached during flood events (mts).

Figure 2. Plant species richness patterns across soil development. **(a)** Mean plant species richness per plot across stage of development. Annual and perennial species richness across soil development in **(b)** sedimentary, **(c)** volcanic-1 and **(d)** volcanic-2 chronosequences. Mean ± SE are given for each stage.

Figure 3. The relative contribution of soil properties on **(a)** annual and **(b)** perennial plant species richness across soil development. Bars in figures represents the first PLSR component with the relative contribution of P_{OLSEN} (white), soil N:P (light gray), soil pH (dark gray), and soil salinity (black) on plant species richness during soil development.

Figure 4. Mean (± SE) for plant-plant interaction intensity (RII) beneath deciduous (open bars) and evergreen (filled bars) shrubs across soil development in **(a)** sedimentary, **(b)** volcanic-1, and **(c)** volcanic-2 chronosequence. Arrow show the direction of soil resource availability and soil non-resource stress.

Figure 5. RII of deciduous (open circles) and evergreen (filled circles) shrubs effects on annual species richness (spp/plot) during soil development in **(a)** sedimentary, **(b)** volcanic-1, and **(c)** volcanic-2 chronosequences.

Figure S1. First axis of Redundancy Analysis (RDA) ordination of annual and perennial composition across soil development in **(a-b)** sedimentary, **(c-d)** volcanic-1, and **(e-f)** volcanic-2 chronosequences. Values in parenthesis indicates variance explained by first

axis of RDA. Significant differences in plant composition among stages of development estimated by PERMANOVA at $p < 0.05$.

Figure S2. Changes in plant forms abundance (ind/plot) across soil development in **(a)** sedimentary, **(b)** volcanic-1, and **(c)** volcanic-2 chronosequences. All abundance data were corrected by plant cover.

Figure S3. Mean (± SE) for plant-plant interaction intensity (I_{IMP}) beneath deciduous (open bars) and evergreen (filled bars) shrubs in **(a)** chronosequences, and across soil development in **(b)** sedimentary, **(c)** volcanic-1, and **(d)** volcanic-2 chronosequence. Arrow show the direction of soil resource availability and soil non-resource stress.

Figure 1.

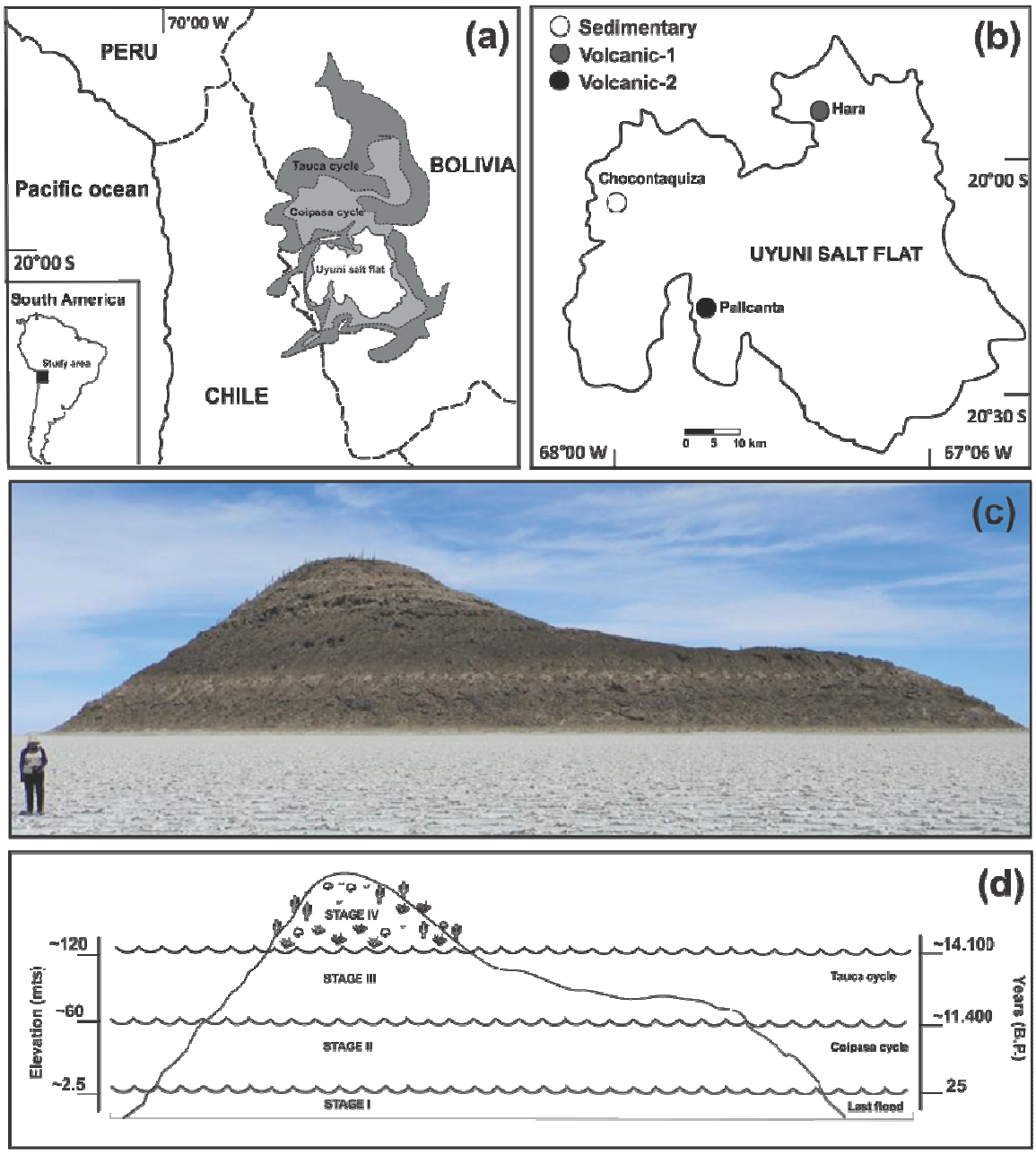

(a)
70°00 W
PERU
Pacific ocean
20°00 S
South America
Study area
Tauca cycle
Coipasa cycle
Uyuni salt flat
BOLIVIA
CHILE
(b)
Sedimentary
Volcanic-1
Volcanic-2
Hara
Chocontaquiza
20°00 S
UYUNI SALT FLAT
Pelicanta
20°30 S
68°00 W
0 5 10 km
67°06 W
(c)
(d)
STAGE IV
STAGE III
STAGE II
STAGE I
~120
~60
~2.5
Elevation (mts)
Tauca cycle
Coipasa cycle
Last flood
~14.100
~11.400
25
Years (B.P.)

Figure 2.

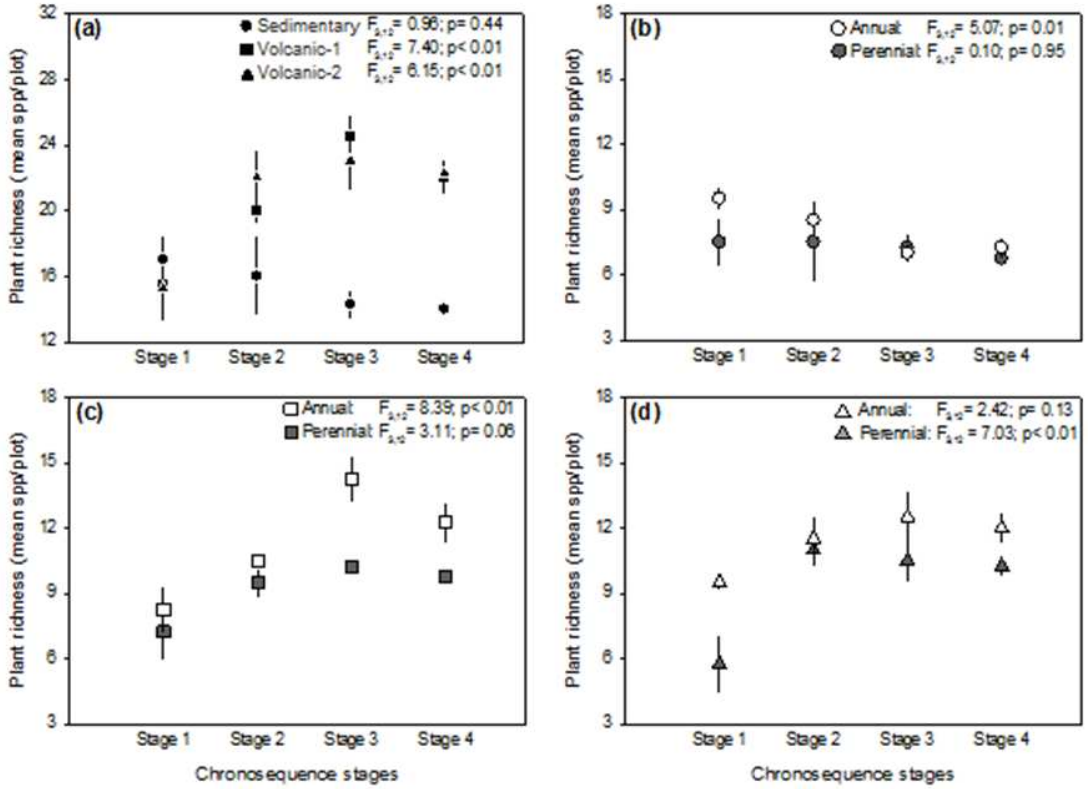


Figure 3.

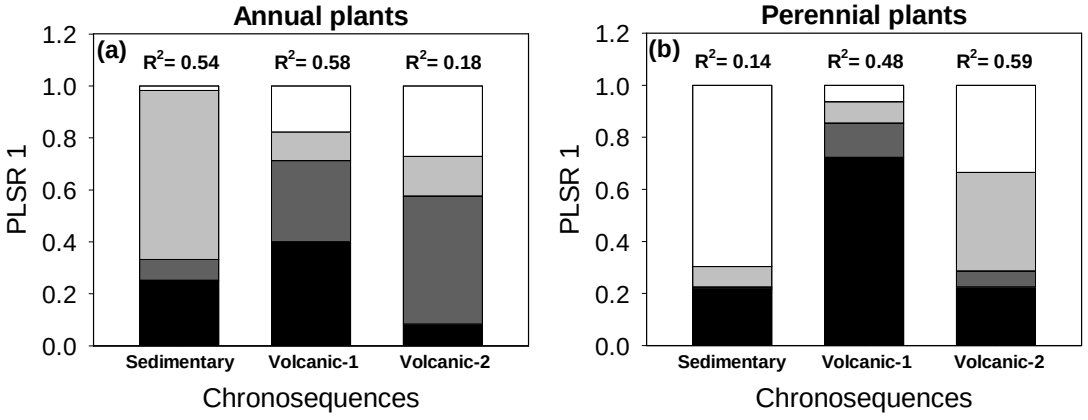


Figure 4.

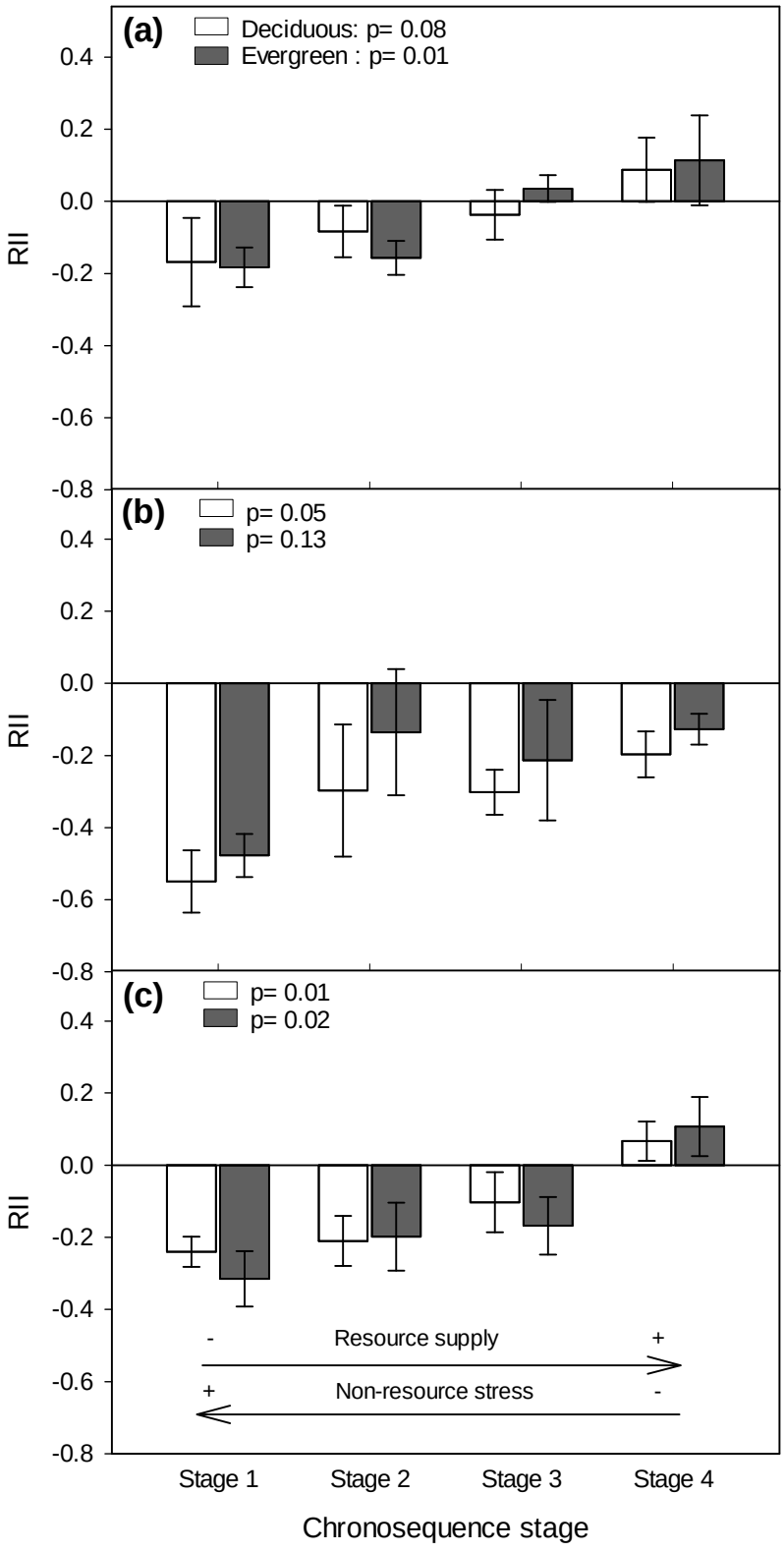

(a)
Deciduous: p= 0.08
Evergreen : p= 0.01
(b)
p= 0.05
p= 0.13
(c)
p= 0.01
p= 0.02
RII
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-
Resource supply
+
+
Non-resource stress
-
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage

Figure 5.

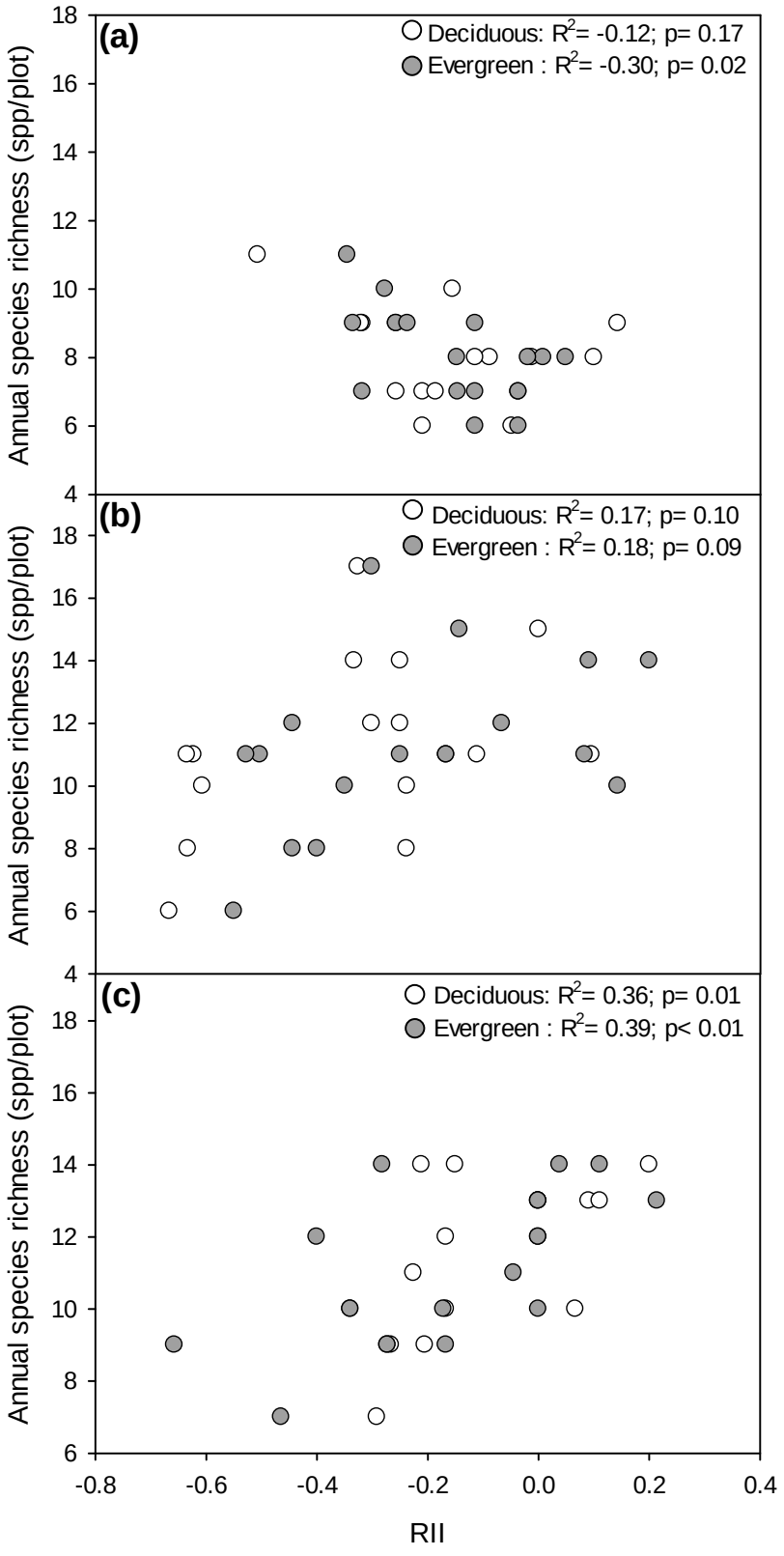

(a)
Deciduous: R2= -0.12; p= 0.17
Evergreen : R2= -0.30; p= 0.02
(b)
Deciduous: R2= 0.17; p= 0.10
Evergreen : R2= 0.18; p= 0.09
(c)
Deciduous: R2= 0.36; p= 0.01
Evergreen : R2= 0.39; p< 0.01
Annual species richness (spp/plot)
RII
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
4
6
8
10
12
14
16
18

Table S1. Species richness and biomass of plants below the canopy of two species of shrubs *Atriplex imbricata* (Dec= deciduous); *Baccharis tola* (Eve= evergreen) and open areas (Gap). Mean (±SE) for each treatment.

Chronosequence	Shrub cover (%)		Treatments								
			Plant richness			Species contribution[1]			Plant biomass (g m^{-2})		
	Dec.	Eve.	Dec.	Eve.	Gap	Dec.	Eve.	Gap	Dec.	Eve.	Gap
Sedimentary	11.12 (1.44)	4.93 (0.69)	3.67 (0.25)	3.67 (0.27)	4.23 (0.28)	2	2	0	2.42 (0.48)	1.81 (0.42)	3.07 (0.60)
Volcanic-1	8.81 (1.19)	4.51 (0.63)	2.73 (0.30)	3.23 (0.30)	5.43 (0.33)	3	2	1	1.78 (0.33)	**1.34** (0.22)	1.82 (0.27)
Volcanic-2	8.12 (2.1)	3.75 (0.75)	3.43 (0.25)	3.43 (0.32)	4.6 (0.27)	5	2	6	**2.63** (0.49)	1.24 (0.21)	**4.35** (0.71)

Values in bold indicate statistical significant differences from one-way ANOVAs across stages of soil development for individual chronosequences.
[1]= species present only on one treatment.

Table S2. Mean of plant species richness per plot and estimation of species richness by Chao-2 and Sørensen coefficient of community similarity across soil development in three chronosequences of Andean Dry Puna, Bolivia. Mean ± SE are given for each stage.

Chronosequence	Plant species richness					
	Annual			Perennial		
	Mean spp/plot	Chao-2	Sørensen	Mean spp/plot	Chao-2	Sørensen
Sedimentary			0.64 (0.04)			0.77 (0.03)
Stage I	9.50 ± 0.50	10.75 ± 0.73		7.50 ± 1.04	11.02 ± 0.07	
Stage II	8.50 ± 0.65	11.37 ± 1.02		7.50 ± 1.85	12.56 ±1.13	
Stage III	7.00 ± 0.41	12.12 ± 0.44		7.25 ± 0.63	14.51 ± 3.19	
Stage IV	7.25 ± 0.48	7.96 ± 0.30		6.75 ± 0.25	8.73 ± 0.12	
Volcanic-1			0.75 (0.03)			0.77 (0.04)
Stage I	8.25 ± 1.03	11.25 ± 0.73		7.25 ± 1.31	11.15 ± 0.50	
Stage II	10.50 ± 0.29	23.25 ± 5.46		9.50 ± 0.65	11.99 ± 1.23	
Stage III	14.30 ± 1.03	30.25 ± 10.35		10.30 ± 0.25	15.25 ± 3.47	
Stage IV	12.30 ± 0.95	17.56 ± 1.13		9.75 ± 0.25	12.75 ± 1.76	
Volcanic-2			0.77 (0.01)			0.75 (0.03)
Stage I	9.50 ± 0.29	18.25 ± 2.67		5.75 ± 1.31	10.15 ± 0.50	
Stage II	11.00 ± 1.47	15.18 ± 0.59		11.00 ± 0.71	19.51 ± 5.49	
Stage III	12.50 ± 1.19	16.27 ± 1.66		10.50 ± 0.96	12.01 ± 0.59	
Stage IV	12.00 ± 0.71	12.99 ± 0.48		10.30 ± 0.48	11.75 ± 0.72	

Figure S1.

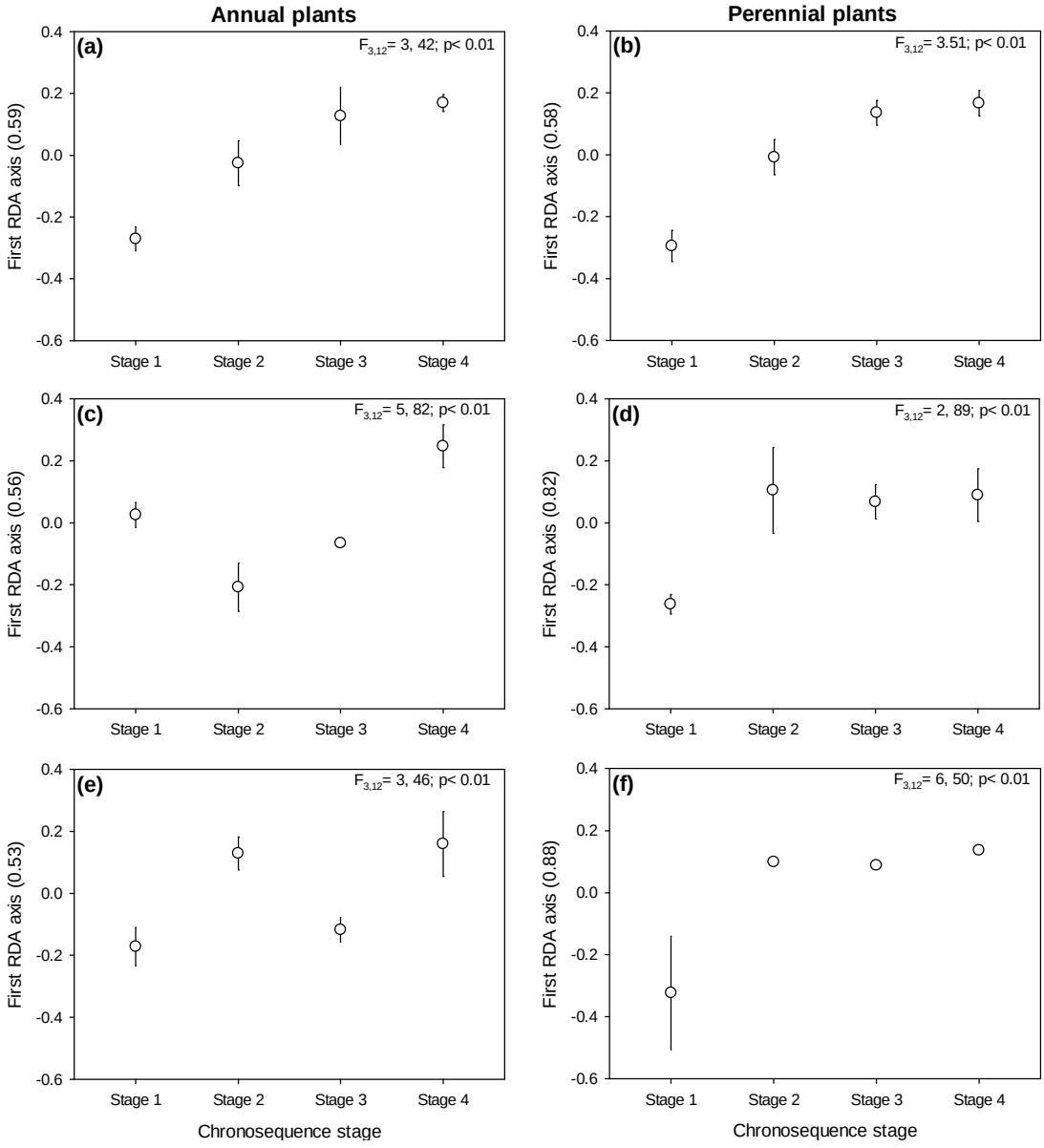

Annual plants
Perennial plants
(a)
$F_{3,12}$= 3, 42; p< 0.01
(b)
$F_{3,12}$= 3.51; p< 0.01
(c)
$F_{3,12}$= 5, 82; p< 0.01
(d)
$F_{3,12}$= 2, 89; p< 0.01
(e)
$F_{3,12}$= 3, 46; p< 0.01
(f)
$F_{3,12}$= 6, 50; p< 0.01
First RDA axis (0.59)
First RDA axis (0.58)
First RDA axis (0.56)
First RDA axis (0.82)
First RDA axis (0.53)
First RDA axis (0.88)
0.4
0.2
0.0
-0.2
-0.4
-0.6
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage

Figure S2.

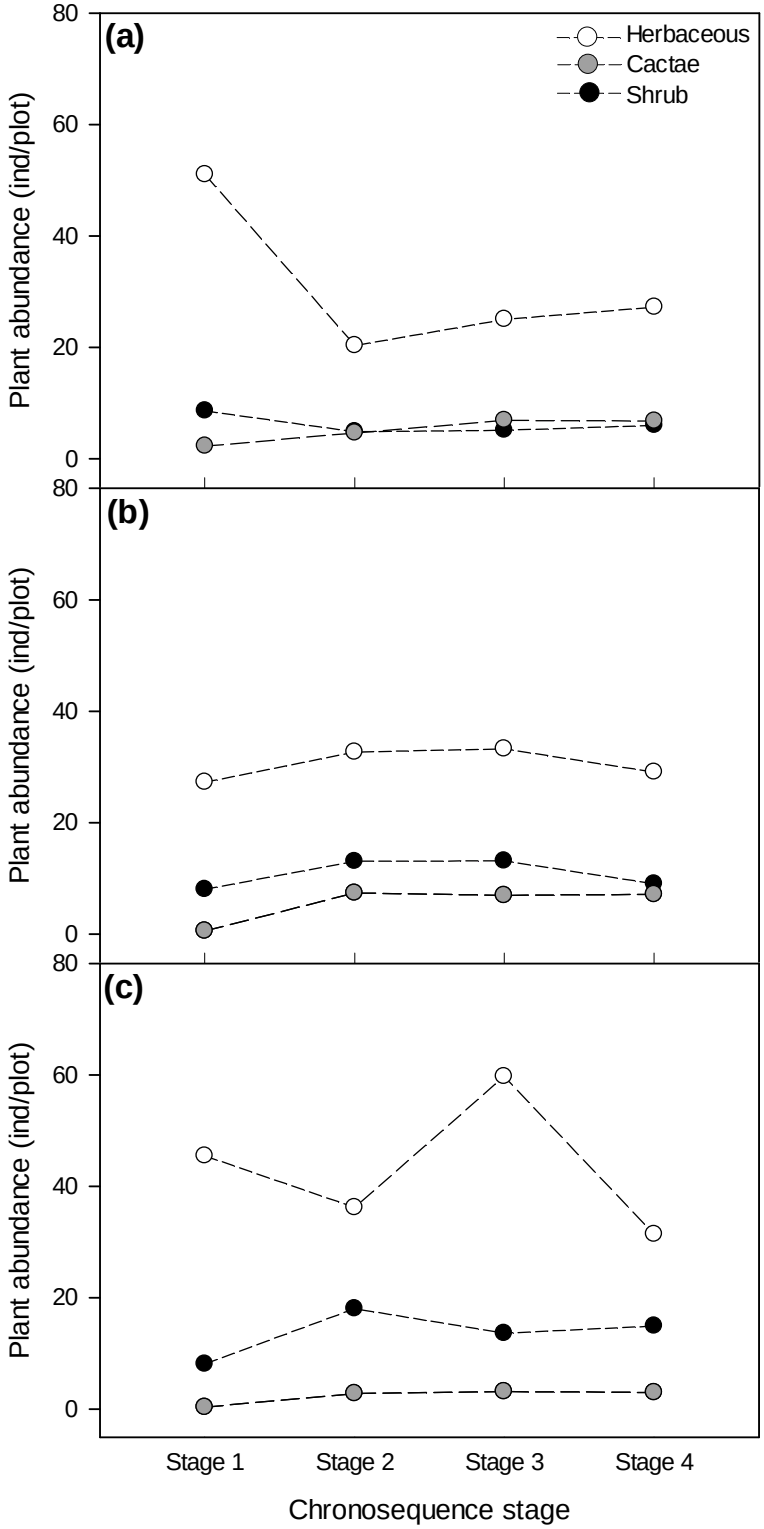
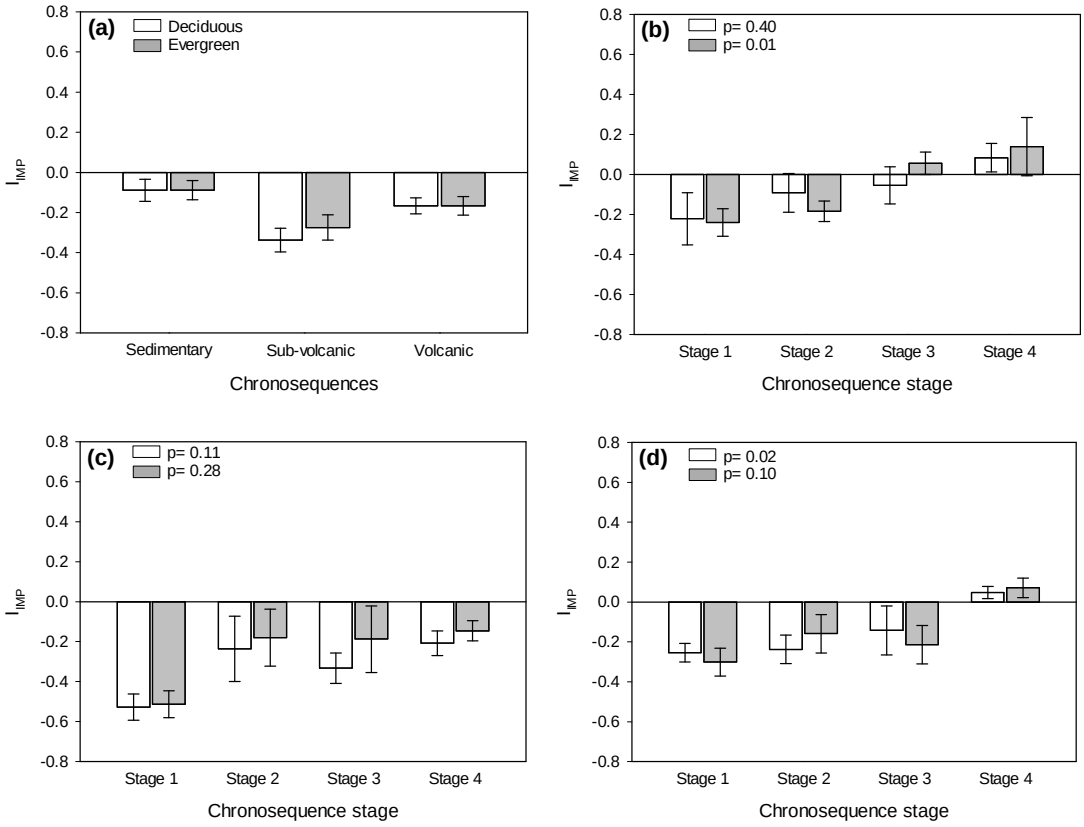

(a)
(b)
(c)
Herbaceous
Cactae
Shrub
Plant abundance (ind/plot)
0
20
40
60
80
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage

Figure S3.


(a)
Deciduous
Evergreen
I_{IMP}
Sedimentary
Sub-volcanic
Volcanic
Chronosequences
(b)
p= 0.40
p= 0.01
Stage 1
Stage 2
Stage 3
Stage 4
Chronosequence stage
(c)
p= 0.11
p= 0.28
(d)
p= 0.02
p= 0.10

CAPÍTULO III

Soil bacterial and fungal communities display contrasting patterns across long-term soil development: evidence from the Andean Dry Puna

Summary

1. As soil development proceed soil biogeochemical properties change affecting the structure and composition of microbial communities. Since bacterial and fungal organisms have different nutrient requirements and metabolic activities, these microbial groups could be differentially affected by shifts in soil properties across soil development. These changes modify microbial dominance and thus, affect ecosystem processes such as decomposition and nutrient cycling during soil development. Thereby, soil development and ecosystem function interactions would be largely dependent of microbial structure and composition.

2. Here, we identified which soil and plant attributes promote the development of bacterial and fungal communities across soil development on soils derived from different parent material in three primary successional c. 20.000-years chronosequences on the Andean Dry Puna, Bolivia. We evaluated the microbial patterns by analyzing the Terminal Restriction Length Polymorphism (T-RFLPs) from amplified bacterial (16S rRNA) and fungal (Internal Transcribed Spacer) genes.

3. Soil bacterial and fungal community composition exhibited contrasting patterns during long-term soil development, although, the magnitude of differences was largely dependent of type of chronosequence. Bacterial composition change as soil pH and plant composition change across soil development in volcanic-1 and volcanic-2 chronosequences, and bacterial communities in sedimentary chronosequence were not affected by soil resource heterogeneity during soil development. Meanwhile, fungal community composition was unresponsive to pedogenesis in all type of chronosequences.

4. Microbial dominance expressed as fungal:bacterial ratio declined across soil development in all chronosequences, with late stages of development showing the lowest

ratios. Decreases in ratios were indicative of increase in bacterial abundance during late stages of development, where soil nutrient content increase and soil conditions are less stressful (low salinity and neutral pH). At difference of fungal composition, fungal abundance change during soil development and this pattern was determined by changes in nutrient pools as soil age increased.

5. *Synthesis and applications*. Our results demonstrate that in this dry and N-poor environment bacterial and fungal communities have different responses to long-term soil development. Although different patterns of abundance and composition were observed among chronosequences with soils derived from distinct parent material, bacterial communities were highly responsive to long-term soil development. These results imply that during long-term pedogenesis shifts in soil properties and concomitant changes in microbial community structure and composition are dependent of type of parent material from which soil derived. We strongly suggest that parent material effect during soil development needs to be explicitly incorporated in chronosequence studies.

Key-words: Andean Dry Puna, soil microbial, chronosequence, soil pH, parent material

Introduction

Long-term changes in soil and ecosystem structure associated with differential concentrations of soil macronutrients like Nitrogen (N) and Phosphorus (P) have been shown to modulate microbial community structure and composition (Kuramae et al, 2001; Vitousek, 2004; Schmidt et al, 2008; Welc et al, 2012).Changes in the relative abundance of bacteria and fungi along soil development have been associated to the fact that microbial groups specialized in the acquisition of nutrient compounds dominate different stages of development (Lambers et al, 2008; Welc et al, 2012). For example, in mesic environments decreases in soil P, associated with soil age, are correlated with increases of fungal biomass (Williamson et al, 2005; Moore et al, 2010). Similarly, increases in soil N with soil stage during soil chronosequences of different glacier forefield were correlated with increases in bacterial abundance (Schmidt et al, 2008; Esperschütz et al, 2011; Göransson et al, 2011). Changes in microbial communities with soil development, are mirrored by plant community composition (Richardson et al, 2004; Wardle et al, 2008), with plants with different life history traits that promote differential nutrient acquisition also occurring in distinct stages of soil chronosequences (Coomes et al, 2005; Wardle et al, 2004a; Lambers et al, 2008). These changes in plant composition have been linked to feedbacks between species with different life strategies and nutrient cycling, which strongly determine the rates of organic matter accumulation and nutrient availability across soil development (Walker and del Moral, 2003; Bardgett et al, 2005; Coomes et al, 2005). Thereby, plant species turnover strongly modulates the structure and composition of soil microbial communities (Marschner et al, 2001; Bardgett et al, 2005) by promoting the dominance of microbial groups with metabolic needs that acquire different nutrient compounds and retain limiting

nutrients (Wardle et al, 2004b; Bardgett et al, 2005). However, how variations in soil chemistry and plant species affect microbial communities during long-term soil development remains unclear (Tarlera et al, 2008; Moore et al, 2010).

Above- and belowground processes during soil development, together with different physiological requirements of fungi and bacteria (Wardle et al, 2004b; Van Der Wal et al, 2006) promote changes in the relative abundance and composition of these microbial groups along stages of soil chronosequences (Bardgett et al, 2005; Williams et al, 2013). Bacterial and fungal communities are the dominant biological groups of soil systems (Whitman et al, 1998; Van Der Heijden et al, 2008) and are the primary consumers of belowground food webs (Wardle et al, 2003). Across long-term soil development changes in aboveground processes, such as replacements of plant species with different functional traits regulate not only soil community assemblages but also the functionality of these communities (Bååth and Anderson, 2003; Bardgett et al, 2005; Rousk et al, 2010). For example, young soils in early stages of soil development tend to be dominated by fast-growing plant species with nutrient-rich leaves and roots that promote rapid decomposition and nutrient cycling (Berendse, 1998; Bardgett et al, 2005; Schmidt et al, 2008). Therefore, young soils often support bacteria-based microbial communities that feed on easily degradable organic compounds produced by early-successional plants (Wardle et al, 2004b; Bardgett et al, 2005). Conversely in old, less productive soils, dominate slow-growing, nutrient-use-efficient plants that produce poor quality litter with complex-carbon and phenol-rich compounds (Wardle et al, 2008; Eskelinen et al, 2009). These compounds promote fungal communities over bacterial communities, as fungi tend to have lower carbon and energy requirements than bacteria (Deubel and Merbach, 2005; Eskelinen et al,

2009; Strickland and Rousk, 2010). Hence, reductions in abundance of bacterial respect to fungal abundance are expected as soil age increase (Esperschütz et al, 2011;Welc et al, 2012).

During soil development as parent material becomes exposed, via the abiotic (e.g. abrasion) and biotic (e.g. mineralization) weathering, soils are formed and secondary minerals are released in soluble forms for plant and microbial nutrition (Anderson, 1988; Huggett, 1998; Lichter, 1998; Yavitt, 2000). As soil development proceeds, the interaction between parent material and soil microbial activity are responsible for bioavailability of substrate-derived nutrients (i.e., Phosphorous), accumulation of organic matter and most processes associated with pedogenesis (Chadwick and Chorover, 2001; Deubel and Merbach, 2005; Esperschütz et al, 2011). Feedbacks between parent material and soil microbial activities are expected to increase in importance as soil age increase (Ulrich and Becker, 2006; Wagai et al, 2011). For example when specialized soil microbial groups facilitate the acquisition and mineralization of adsorbed and immobilized N and P, which in the absence of disturbances, remained bounded in organic forms of difficult accessibility for plants (Cornelissen et al, 2001; Lambers et al, 2008; Wagai et al, 2011). Thus, the relative importance of different soil microbial communities and feedbacks between above- and belowground process could be strongly modulated by parent material (Marschner et al, 2001; Ulrich and Becker, 2006; Moore et al, 2010).

Accordingly, studies from soil chronosequences in tropical and temperate ecosystems than span from sand-dunes (Laliberte et al, 2012), lava flows (Crews et al, 1995), glacier retreats (Walker and Syers, 1976; Richardson et al, 2004) to sequences of marine terraces (Moore et al, 2010), have shown that transition from N to P limitation along

soil development are associated to changes in ecosystem processes such as decomposition, nutrient cycling and primary production (Peltzer et al, 2010), besides, of shifts in plant and microbial community composition (Coomes et al, 2005; Jangid et al, 2013); thereby, emphasizing aboveground-belowground linkages during soil development. Furthermore, we recently showed that in high altitude dry Andean ecosystems, soil changes in soil N and P availability do not mirror those described for mesic ecosystems even after 20,000 years of soil development (Alfaro et al, Chapter I). Extreme slow soil development determined by low soil N content was observed in three chronosequences with contrasting geological origin, although differences in the magnitude of these effects were determined by parent material origin. Indeed, we reported that increases in soil N and decreases in soil pH drove plant individual (e.g. leaf and litter chemistry) and community (e.g. composition) changes during soil development, as well as ecosystem processes such as litter decomposition. These patterns were markedly different among chronosequences with soils derived from distinct parent material. Therefore, we expected strong feedbacks between the structure and composition of soil microbial communities and parent material during soil development.

We used a metagenomic approach to examine soil bacterial and fungal community patterns across stages of soil development in chronosequences. Specifically we hypothesized: (1) that differences in soil and plant patterns during soil development among chronosequences with soils derived from distinct parent material will affect the composition and productivity of microbial communities (bacterial and fungal) along soil development. In particular (2) that soil fungal:bacterial ratio (as a measure of microbial biomass) will decline as soil age proceeds, due to increases in bacterial abundance associated to higher soil nutrient contents (notably N), and decrease in alkalinity of soils

(decline soil pH) during late stages of development, (3) that bacterial and fungal community composition will change across soil development in response to changes in soil quality from early alkaline and nutrient poor soils to neutral soils with high nutrient availability (high N:P ratio) during late stages of development, and (4)that increases in plant litter nutrient quality through their effects on soil nutrient availability will determine increases in bacterial over fungal abundance during soil development.

Materials and methods

STUDY SITES

The chronosequence system is located in the Uyuni salt flat (Uyuni-SF) in the Central Andean Dry Puna of Bolivia (Alfaro et al, Chapter I). The climate is dry and cold most of the year (Vuille et al, 2000), with mean annual temperature around of 8.5 °C, and mean annual precipitation of 176 mm. Superficial soils in the chronosequences are sandy in texture with low organic matter content (Urcelay et al, 2011)and extensive deposits of tufa (Placzek et al, 2009). All chronosequences are discrete units like elevated "islands" surrounded by a large saline matrix (Alfaro et al, Chapter I) and in this study we chose to work on chronosequences with different parent material; sedimentary deposits, and two different volcanic rocks forming Andesitic-basaltic domes and Dacitic domes (SGM, 1995; SGM, 2002; Tibaldi et al, 2009). The substrate age gradient originated as a consequence of changes in the precipitation regime causing repeated advance and retreat of the water level across the Uyuni basin over last 18·000 years (Alfaro et al, Chapter I).

SAMPLING

At all three chronosequences, we set up four plots (30 x 2 m) at each of the four stages of soil development. All plots were arranged with a west-facing exposure. Soil samples and plant litter were collected at the end of the rainy season (early May) when primary productivity is highest. At each plot, we collected soils from three randomly assigned points from the 0-10 cm layer; these subsamples were pooled and homogenized into a single sample, after half of each homogenized sample was used for microbial community analysis and for this we store all samples at -80°C and sent them to the Cellular Ecology Laboratory (Pontificia Universidad Católica de Chile) for molecular analyses. The rest of soil samples were stored in plastic bags at -4°C to laboratory analysis. After to collect soil samples Whatman filter papers were buried in the space left by the removed soil to evaluated decomposition. The results of soil and plant chemical analysis and detailed methodology for evaluation of plant cover, plant community composition and decomposition experiments in Alfaro et al (Chapter I).

METAGENOMIC ANALYSIS

DNA extraction and Terminal Restriction Length Polymorphism (T-RFLP)

In order to evaluate differences in fungal and bacterial composition across stages of soil development of the three chronosequences we analyzed the T-RFLP from amplified (Polymerase Chain Reaction, PCR) bacterial and fungal 16S rRNA and Internal Transcribed Spacer (ITS) genes respectively. DNA was extracted from 0·5 g of soil using the FastDNA® SPIN Kit for Soil and the FastPrep® instrument (MP Biomedical, LLC, Solon, OH, USA), according to the manufacturer's instructions. The quantity of extracted DNA was determined using a Nano-Drop spectrophotometer (NanoDrop Technologies).For

T-RFLP analysis, bacterial and fungal 16S rRNA genes were amplified with the primers 8F (5 end labeled with FAM fluorescent dye) and 1392R (Lane et al, 1985), and ITS1-F (5 end labeled with FAM fluorescent dye) and ITS4 (Gardes and Bruns, 1993) respectively. The PCR mixture (50 μL) contained 1 μL of template DNA, 49 μL of 10× reaction buffer, 33 μL of MgCl2, 11 μL of dNTP, 22 μL of each forward (f) and reverse (r) primer (0·2 mM), and 1·0 U of Taq DNA Polymerase for bacteria and fungi (both with specific primers). PCR cycling was performed as follows: 5 min at 94°C, followed by 25 cycles of 94°C for 45s, 56°C of annealing temperature for 45s, 72 °C for 2 min, and a final extension step at 72 °C for 7 min. for bacteria analyzes. For fungi analyzes 7 min at 95 °C, followed by 4 cycles of 95°C for 40s, 49°C for 30s, 72°C for 48s and 31 cycles of 94°C for 40s, 54°C for 30s, 72°C for 48s, and a final 7 min extension step at 72°C. PCR products were digested with the enzymes MspI and HhaI and fungi was digested with enzymes TAQ1. After 20uL of digestion PCR product from each sample was sequenced by Macrogen (Seuol, Korea). In this sequenced analysis the peaks represent the size of Terminal restriction fragments (TRFs), and the peak areas represent the relative proportion of these fragments. Following previous works TRFs from 50 to 700 bp were included in analyses, and those representing less than 0·5% of the total area were excluded.

Microbial biomass quantification

We quantified bacterial biomass following Einen et al (2008) and fungal biomass following Prévost-Bouré et al (2011) protocols. The microbial qPCR were performed using (kits) and were running on qPCR EcoTM Real-Time (Illumina®) machine. Details of the primers and thermal profile used are in supplementary material (Table S5). To estimate the bacterial and

fungal biomass the number of 16S and 18S rRNA copies ng^{-1} of DNA obtained from qPCR analysis was converted to a number of rRNA copies g^{-1} of dry soil.

STATISTICAL ANALYSIS

We evaluated if microbial composition changed across stages of soil development using multivariate distance-based ordination analyses. Square-root (Hellinger transformation) transformed data (Legendre and Gallagher, 2001) was used to calculate Bray–Curtis dissimilarity matrices (Legendre and Gallagher, 2001). Non-Metric Multidimensional Scaling (NMDS) was performed to graphical ordinate bacterial and fungi community composition across stages of soil development. Two-way analysis of similarity (ANOSIM) was performed to test differences in soil microbial composition across stages and chronosequences. The test statistic, R, in ANOSIM ranges from 0 to 1, where values near 0 indicate no difference between groups, whereas values close to 1 signify high dissimilarity between groups (Rees et al, 2004).

We compared microbial community biomass among stages of soil development using one-way ANOVAs and pairwise differences were assessed with Tukey's HSD (honestly significant difference) post hoc test. Fungal:Bacterial ratio was used as a measure of relative change in fungal to bacterial dominance across soil development (Fierer et al, 2009). All data were tested for homogeneity of variances and transformed if necessary to meet ANOVA assumptions. Pearson's correlations between the first axis score of the NMDS ordination of bacterial and fungal community composition and soil and plant variables were conducted to evaluate the effect of soil and plant characteristics on microbial composition along soil development. Mantel tests were performed to evaluate the influence of spatial distance (among sampling plots)and soil and plant variables on microbial

biomass. We used partial Mantel test to examine the effect of geographic distance (straight-line distance between sampling plots) on community composition correcting for variation in soil and plant characteristics. The environmental similarity matrix was estimated from Euclidean distances for all soil and plant variables (e.g. pH-distance). These analyses were carried out with PRIMER-E 5 software (Clarke and Gorley, 2006). Statistical analyses were performed using Statistica 9·0 (StatSoft Inc., Tulsa, Oklahoma, USA) and R (vegan) ver. 2·9·1 (Development Core Team 2009).

Results

BIOTIC AND ABIOTIC PROPERTIES DURING SOIL DEVELOPMENT

Soil and plant characteristics were largely different among the three types of chronosequences (Table 1). For the twelve environmental variables considered, we found significant differences among chronosequences in eight (p values in Table 1). However, some soil and plant characteristics changed in similar way during soil development in three chronosequences, such as the increase in soil N and N:P ratio; and the declining in soil salinity and soil pH. While plant cover and litter C:N declined in all chronosequences, plant species richness increased across soil development in volcanic-1 and volcanic-2 chronosequences. Total C, total P and P_{OLSEN} values, despite their variations among stages of soil development in some chronosequences, did not show any clear pattern (Table 1).

SOIL MICROBIAL PATTERNS AMONG CHRONOSEQUENCES

Fungal:bacterial ratios were significantly different among chronosequences, with sedimentary chronosequences showing lowest values (Fig. 2a). These differences were determined by lower fungal abundance in sedimentary chronosequence and lower bacterial

abundance of volcanic-1 chronosequence (Table 2). Nevertheless the large differences in soil properties among chronosequences (Table 1), soil factors were mostly weak and not significantly related to variation in fungal:bacterial ratio (Table S4).

On the other hand, bacterial community composition (BCC) was significantly affected by type of chronosequence as bacterial communities from all types of chronosequences were significantly different ($p< 0{\cdot}01$; global $R= 0{\cdot}42$; Table S2). Bacterial communities from volcanic-1 chronosequence were largely different from communities of sedimentary ($R= 0{\cdot}55$; $p< 0{\cdot}01$) and volcanic-2 chronosequences ($R= 0{\cdot}58$; $p< 0{\cdot}01$). Ordination analyses showed that bacterial communities from all three chronosequence were clearly distinguishable, with first axis of NMDS explained 66% of variation in composition (Fig. 3a). These differences in bacterial community composition among chronosequences were associated to differences in soil nutrients such as N ($r= 0{\cdot}28$; $p= 0{\cdot}04$), P ($r= 0{\cdot}29$; $p= 0{\cdot}04$) and P_{OLSEN} ($r= 0{\cdot}49$; $p< 0{\cdot}01$) and plant community composition ($r= 0{\cdot}30$; $p= 0{\cdot}03$). In contrast, fungal community composition (FCC) did not differ among chronosequences ($p< 0{\cdot}83$; the global $R= 0{\cdot}01$; Table S2). Indeed, compositional ordination analysis showed that fungal communities from all three chronosequences were overlapping, indicating strong similarity (Fig. 3b).

SOIL MICROBIAL PATTERNS ACROSS SOIL DEVELOPMENT

i) Sedimentary chronosequence

Soil bacterial abundance increased across stages of the sedimentary chronosequence from $5{\cdot}54E+07$ to $13{\cdot}62E+07$ gene copies g^{-1} soil. In contrast, fungal abundance declined from $10{\cdot}11E+06$ to $6{\cdot}47$ E+06gene copies g^{-1} soil. Accordingly, fungal:bacterial ratios decreased with soil age (Fig. 2b) and this patterns was associated to changes in soil C ($r= 0{\cdot}40$; $p=$

0·04) and soil P (r= 0·29; p= 0·03). As expected declining in soil pH and plant litter C:N across soil development promoted bacterial abundance (r= -0·83, p= 0·01; Table S1) which in turn contributed to decrease fungal:bacterial ratio during late stages of soil development. Instead, declining in fungal abundance as soil age increased was mainly associated to changes in nutrient pool such as C:N (r= 0·70, p= 0·01) and plant community composition (r= -0·66, p= 0·01) during soil development.

Bacterial and fungal composition turnover across soil development was low and not significant (Fig. 3c, 3d). ANOSIM measures of community differentiation (R values) among stages of soil development were very low in this chronosequence (Table S3). Even pairwise comparisons between adjacent stages (e.g. stage 1 and stage 2) were not significant different (Table S3). Thus, in this chronosequence, patterns of microbial community composition were not affected by soil pedogenesis

ii) Volcanic-1 chronosequence

In this chronosequence bacterial abundance increased during late stages of development from 2·63E+07 to 5·42E+07 gene copies g^{-1} soil(Table 2) and the same pattern was observed for fungal abundance, which increased from 1·03E+07 to 1·22E+07 gene copies g^{-1} soil. Both bacterial and fungal groups increases were related to change in soil pH from alkaline young stages to neutral soils in late stages of development (r= -0·78; p< 0·01; r= -0·60; p< 0·01). Additionally, bacterial abundance increases also was determined by improve in soil conditions across soil development associated to declining in soil salinity (r= -0·50; p= 0·03) and plant litter C:N (r= -0·67; p= 0·02). Even that both groups increased across soil development, large increase in bacterial abundance determined that

fungal:bacterial declined during late stage of development (Fig. 2c). Mantel tests coefficient showed that fungal:bacterial ratios decreases was associated to changes in soil moisture (r= 0·25; p= 0·04) and plant litter C:N (r= 0·26; p= 0·04).

Graphical ordination across the first NMDS axis and ANOSIM separated the BCC according to the four stages of soil development (R= 0·37; p< 0.01; Fig. 3e). In this chronosequence, bacterial communities from first stage were largely different to communities in third (R= 0·58; p< 0.01) and fourth (R= 0·60; p< 0.01) stage of development (Table S3). Soil pH was largely the most important soil factor determining the bacterial community turnover across soil development (r= 0·72; p= 0·01; Fig. S1). In contrast, ANOSIM indicated that fungal composition not changed across soil development (R= 0·05; p= 0·72), indeed, graphical ordination of NMDS showed that communities of all stages were highly overlapped (Fig. 3f).

iii) Volcanic-2 chronosequences

Both microbial groups declined in abundance along stages of development in this chronosequence (Table 2). Bacterial changed from 7·39E+07 to 4·78E+06 gene copies g^{-1} soil and, fungal abundance declined from 1·69E+07 to 4·74E+05 gene copies g^{-1} soil during soil development. In contrast to the sedimentary and volcanic-1 chronosequences, in this chronosequence bacterial abundance showed a declining across soil development, and this contributed that decrease of fungal:bacterial ratio was not significantly across soil age gradient ($F_{3,12}$= 1·30; p= 0·32; Fig. 2d). Indeed, fungal to bacterial ratio declining during soil development was not significant related to any soil of plant changes. While, bacterial and fungal declining across soil development was affected by the same factors such as

decrease in plant cover (r= 0·60; p= 0·02; r= 0·60; p= 0·04), soil pH (r= 0·53; p= 0·01; r= 0·53; p= 0·04) and change in soil P_{OLSEN} (r= 0·57; p= 0·04; r= 0·47; p= 0·03).

Bacterial community composition was indeed strongly affected by soil pedogenesis (R= 0·38; p< 0·01), with communities from stages clearly differing across the first axis of NMDS ordination (Fig 3g). Bacterial communities from first stages of development were largely different to communities in third (R= 0·69; p< 0·01) and fourth (R= 0·60; p< 0·01) stages of development (Table S3). At difference of sedimentary chronosequence changes in BCC was associated with change in multiple soil and plant variables, such as increase in soil N (r= 0·49, p= 0·03) as well as declining in P_{OLSEN} (r= -0·51, p= 0·03), soil moisture (r= -0·53, p= 0·02), soil salinity (r= -0·51, p= 0·04) and changes in plant composition (r= 0·54, p= 0·01). Likewise occurred in sedimentary and volcanic-1 chronosequences fungal community composition was not affected by pedogenesis (R= 0·13; p= 0·12; Fig. 3h).

MICROBIAL BIOMASS AND DECOMPOSITION

In the Uyuni-SF, although decomposition increased significantly across soil development in all chronosequences, these patterns were differentially related to changes in microbial abundance on each chronosequence. At difference of sedimentary chronosequence, bacterial and fungal abundance changes were associated to shifts in decompositions across soil development in volcanic-1 and volcanic chronosequences (Fig. 4). Increases in decomposition during pedogenesis in volcanic-1 chronosequence was positively related to increase in bacterial (r_s= 0·52; p= 0·03) and fungal (r_s= 0·53; p= 0·03) abundance (Fig. 4d). Meanwhile, the declining in bacterial (r_s= -0·64; p<0·01) and fungal (r_s= -0·74; p<0·01) abundance was negatively related to increase of decomposition during soil development in volcanic-2 chronosequence (Fig. 4f). When changes in decomposition were related to

changes in fungal to bacterial ratio a negative effect was observed across soil development in all chronosequences, although, these effects only were marginally significantly in volcanic-1 chronosequences (Fig. 4a, 4c, 4e).

SPATIAL STRUCTURE AND DISTANCE DECAY RELATIONSHIPS (DDR)

As expected across soil development larger differences in BCC occurred between non-contiguous stages, than in contiguous stages, suggesting that geographic distance (average 286 m), would drive the changes in community composition. However, most of bacterial and fungal composition changes were independent of geographic distance (Fig 5a, 5d), even after controlling the effect of environmental variability (Partial Mantel test). Instead, when microbial community similarity was related with variability of soil and plant factors expressed as distance-measures (Euclidean distances of values among plots), soil pH and plant community composition were important predictors of microbial dissimilarity, in particular, in bacterial communities. There was a negative relationship ($r= -0{\cdot}35$, $p< 0{\cdot}01$; $r= -0{\cdot}18$, $p< 0{\cdot}01$) between bacterial dissimilarity and soil pH-distance across volcanic-1 chronosequence (Fig. 5b), suggesting that soils with extremes values of pH have similar bacterial communities across soil development. Whereas, plant composition dissimilarity was positively related with changes in bacterial community differentiation across soil development ($r= 0{\cdot}24$, $p= 0{\cdot}02$; $r= 0{\cdot}19$, $p= 0{\cdot}02$) in volcanic-1 and volcanic-2 chronosequences. These lasts results strongly suggest that particular assembles of plants are associated to particular sets of bacterial communities. On the other hand, low fungal turnover across successional stages was negatively influenced by soil pH-distance in volcanic-2 chronosequence (Fig. 5e)

Discussion

MICROBIAL COMMUNITY PATTERNS ACROSS SOIL DEVELOPMENT

The major objective of our study was to evaluate the relative importance of parent material on soil microbial patterns during soil development. In previous work using the same chronosequences we showed that different parent material, among other things, can increase differences in chemical properties of soils during soil developed under similar soil formation conditions (Alfaro et al, Chapter I), affecting individual traits and community plant patterns, such as nutrient content in leaf and litter, and plant community composition and diversity (Alfaro et al, Chapter I and II). Here we found that soil bacterial and fungal communities display contrasting patterns across soil development, and as expected different environmental factors that covariate with soil age such as soil and litter N content, soil pH and plant community composition were involved on microbial patterns during soil development in each chronosequence.

SOIL MICROBIAL BIOMASS DURING SOIL DEVELOPMENT

Along the Uyuni-SF soil conditions were improving as soil age increased by increase in soil nutrient content (e.g. N), and decrease in soil salinity and soil pH. These patterns, as expected, affected soil microbial dominance across soil development, in particular in sedimentary and volcanic-1 chronosequences, where soil pH was the most important soil factor effecting increase in bacterial abundance during late stages of development. These shifts from fungal to bacterial dominance across soil development has been suggest to be related to different responses to soil pH variation between microbial groups (Bååth and Anderson, 2003; Fierer and Jackson, 2006). Unlike fungi, soil bacterial abundance is particularly sensitive to changes in soil pH due to the effect of this soil factor on optimal

growth rate of prokaryotes (White, 2000; Bååth and Anderson, 2003; Rousk et al, 2010) and, because soil pH affects the reaction capacity and availability of some essential nutrients (e.g. Mg, N, P) for bacterial development (Kemmitt et al, 2006; Kuramae et al, 2011). Thus, neutral soils observed during late stages of development will favor bacterial abundance and will promote the decline in fungal:bacterial (Bååth and Anderson, 2003; Rousk et al, 2010).

Fungal to bacterial dominance along soil development represents an indirect measure of soil conditions (e.g nutrient availability) and the microbial-based food web (Williamson et al, 2005; Van Der Wal et al, 2006; Eskelinen et al, 2009). The increase in bacterial dominance generally is related to changes in above- and belowground conditions (Bardgett et al, 2005) that promote the increase in production of high-quality organic matter (Eskelinen et al, 2009), high nutrient availability (Van Der Heijden et al, 2008) and high rates of soil processes (Wardle et al, 2004b). According to these observations, in the Uyuni-SF the increase in bacterial abundance across soil development was negatively related to plant litter C:N in sedimentary and volcanic-1 chronosequences, with bacteria increasing in abundance as litter quality (N concentration) increased. While fungal abundance was mostly negatively correlated or not related to increase in soil N and N:P across soil development in all chronosequences. Differences in stoichiometric and physiological requirements between bacterial and fungal (Strickland and Rousk, 2010) could explain the different patterns across soil development. In particular, because C:N of bacteria biomass is expected to be lower (~ 3-9) with respect to C:N of fungi (~ 6-17) (Strickland and Rousk, 2010), thereby, fungal biomass are often expected to have lower N requirements (Güsewell

and Gessner, 2009) and lower stoichiometric control relative to bacteria (Van Der Heijden et al, 2008; Strickland and Rousk, 2010).

Since our study was the first in examined changes in bacterial and fungal abundance during long-term soil development, it was difficult to make comparisons of patterns observed. However microbial abundance patterns from a few long-term previous studies indicates that fungal to bacterial ratio increases during soil development was related to large soil N:P and C:P ratios, indicatives of P limitation in late stages of development (Williamson et al, 2005; Moore et al, 2010). These patterns are opposite to observed in the Uyuni-SF and are reflecting that, at difference of our chronosequences, the dominance of fungal-based food webs are determined by increasing P limitation during late stages of development (Williamson et al, 2005). While, evidence from short-term chronosequences (< 1,000 years of development) suggest that fungal and bacterial abundance patterns reported here followed expected changes across soil development (Esperschütz et al, 2011; Welc et al, 2012). Indeed, values of microbial abundance after only hundreds of years of soil development in chronosequences from retreated glaciers (Esperschütz et al, 2011; Göransson et al, 2011) and land abandonment (Van Der Wal et al, 2006) are similar to observed in chronosequences from the Uyuni-SF. These observations reinforce our general idea that extreme slow soil development in the Uyuni-SF chronosequences will be accompanied by slow microbial development. Despite of differences in environmental factors driving changes in bacterial to fungal dominance across soil development among chronosequences with different parent material, lower fungal:bacterial ratio during late stage of development in all chronosequences, suggest that microbial productivity across soil development follow predictable patterns related to slow soil pedogenesis in the Uyuni-SF.

MICROBIAL BIOMASS AND ECOSYSTEM FUNCTION

Soil microbial communities play fundamental roles to nutrient cycling through decomposition across soil development (Wardle et al, 2003, Deubel and Merbach, 2005; Schulz et al, 2013). Therefore, shifts in dominance of microbial groups during soil development are often related to changes in decomposition rates (Williamson et al, 2005). In the Uyuni-SF microbial abundance and decomposition showed different relationships across soil development on each type of chronosequence. As expected, increase in bacterial abundance during late stages of development was associated to high decomposition rates in sedimentary and volcanic-1 chronosequences. These patterns are similar to reported by Williamson et al (2005) that suggest that bacterial-based food webs are related to high decomposition. In contrast, high fungal:bacterial ratio during early stages of development in all chronosequences indicated that fungal communities dominated highly alkaline and nutrient poor soils (low N) where decomposition was lower. These observations strongly suggest that youngest stages during soil development are dominated by groups with low biomass turnover, highly nutrient conservation strategies and lower rates of mineralization and decomposition (Wardle et al, 2004b; Williamson et al, 2005; Schulz et al, 2013).

SOIL MICROBIAL COMPOSITION DURING SOIL DEVELOPMENT

Along the Uyuni-SF the overall observation that fungal community composition (FCC) was mainly homogenous, and that the BCC was widely different among successional stages, suggests that unlike the fungal communities, bacterial composition was more sensitive to the effect of soil and plant variability across soil development, in particular in bacterial communities from volcanic-1 and volcanic-2 chronosequences. Previous studies have found that microbial communities changes across soil development (Williamson et al, 2005), in

particular, large differences among successional bacterial communities are observed in long-term chronosequences (Tarlera et al, 2008; Jangid et al, 2013). These compositional changes often are determined by decrease in soil P and changes in soil pH (Tarlera et al, 2008; Kuramae et al, 2011; Jangid et al, 2013). However in the Uyuni-SF different abiotic and biotic factors drive changes in bacterial composition across soil development among chronosequences with different geological origin.

Orderly succession of bacterial composition (based on graphical ordination of NMDS analysis) along successional stages in volcanic-1 and volcanic-2 chronosequences strongly suggested that bacterial composition changes in a predictable way across soil development, and more importantly, would be revealing the effect of changes in environmental conditions on BCC as soil age increase. For example, the differences in BCC among successional stages in volcanic-1 chronosequence were largely predicted by soil pH decrease. This observation supports previous suggestions that soil pH is the most important predictor of bacterial community composition at different spatial scales (Fierer and Jackson, 2006; Kemmitt et al, 2006; Fierer et al, 2009). This close relationship between soil pH and soil bacterial patterns is probably due to the significant effect of pH on development of prokaryotes (White, 2000; Bååth and Anderson, 2003), apparently determined because these organisms have narrow pH ranges (usually 6·5-7·5) where the growth is maximized (Rousk et al, 2009). Evidence from long-term chronosequences suggests that changes in soil nutrient availability are correlated with a decrease in soil pH (soil acidification) as soils age (Rousk et al, 2009). However, the decline in soil pH across the volcanic-1 chronosequence was only correlated to changes in plant species richness.

As soil development proceeds in volcanic chronosequences, the shift in BCC was related to changes in plant community composition. Our results are consistent with previous studies which highlight that changes in aboveground plant community composition affect soil microbial community composition and structure (Eskelinen et al, 2009; Zinger et al, 2011) through the quality and quantity of resources entering the soil (Wardle et al, 2004b; Güsewell and Gessner, 2009). In long-term chronosequences as soil age increases the soil nutrient limitation (generally soil P) promotes shifts in plant traits that increase the strategies to conserve essential nutrients from senescent tissue (Richardson et al, 2004); and community composition changes (Walker and del Moral, 2003; Zak et al, 2003) that favor species and that retain and use nutrient most effectively (Richardson et al, 2004). Plant species turnover across soil age gradient likely drive soil microbial processes through producing organic matter that differ in chemical composition and quality (Wardle et al, 2008; Eskelinen et al, 2009) and modifying the rhizosphere soil (Marschner et al, 2001). Thereby, changes in microbial composition could track shifts in plant community composition (Berendse, 1998; Kardol et al, 2007), while large differences in soil nutrients across volcanic-2 successional stages did not explain, the BCC changes.

As expected across soil development the larger differences in BCC occurred between non-contiguous stages, suggesting that geographic distance (average, 286 m), would drive changes in community composition (Green and Bohannan, 2006). However, on sedimentary and volcanic-1 chronosequences the BCC patterns were largely independent of geographic distance, even after controlling the effect of environmental variability, and in volcanic chronosequences the effect of geographic distance disappeared after to controlling by soil pH differences. Similar to previous studies geographic distance

was a poor predictor of decay in community similarity (Green and Bohannan, 2006), reflecting the dominant effect of soil and plant characteristic during soil development on microbial community patterns (Berendse, 1998; Lauber et al, 2008; Kuramae et al, 2011).

Concluding remarks

By controlling the climate, soil age and perturbation type our study is the first to examine the relative importance of different environmental factors that drive microbial community patterns across long-term soil development in chronosequences with different parent material. Even large differences in soil and plant patterns during soil development among chronosequences, fungal:bacterial ratio declined in all chronosequences, although different factors determined these patterns on each chronosequence. The response of bacterial communities composition to soil age gradient were widely different those showed by fungal communities across soil age gradient in volcanic-1 and volcanic-2 chronosequences, while microbial (bacterial and fungal) composition was largely invariant across soil development in sedimentary chronosequence. Although, relative importance of environmental factors on bacterial and fungal patterns was different among chronosequences with distinct parent material, the general observation was that soil pH and plant community composition were major drivers of change in bacterial composition across soil development in the volcanic-1 and volcanic-2 chronosequences in the Uyuni-SF.

Together these findings represent several lines of evidence suggesting that bacterial and fungal communities have distinctive successional patterns, and are consistent with the general framework that highlight the importance of environmental heterogeneity for changes in bacterial community composition across soil development and strongly suggest

that parent material could have an critical effect on trajectory of abiotic and biotic components during long-term soil development.

Finally, sustained increase of biogenic elements (i.e soil N) and the decline in soil pH from highly alkaline (pH ~9) to neutral (pH ~ 7) soils across soil development strongly suggest that in our system the all four stages of each chronosequence are in the lower extreme of initial phase of soil development (build-phase) (*sensu* Wardle et al, 2004a). Further, these soil patterns were determinants of compositional and quantitative changes in microbial communities. In fact, the decrease in fungal:bacterial across soil chronosequences are indirect evidence of slow ecosystem development even the large soil age gradient (~20,000 years) in these Andean ecosystems.

Acknowledgements

We thank to Paola Villarroel, Daniela Rivera, Huber Villca, Alejandro Coca and Mauricio Torrico for helping with field and lab work. We thank to Andy Rominger and Fernanda Salinas for their useful comments on earlier drafts of our manuscript. We are very grateful with Center of Biodiversity and Genetics (CBG) for logistic support for fieldwork. F.D. Alfaro was funded by a doctoral scholarship from CONICYT AT-24100099 and Russell E. Train Education for Nature Fellowships. This project was funded by ICM P05-002 and CONICYT PFB-023.

References

Alfaro, F.D., Gaxiola, A. & Marquet, P.A. (2013) Hyper slow soil development after catastrophic disturbance: soil N-limitation is the source of eternal youth in long-term successions in the Central Dry Andes. *In press*.

Alfaro, F.D., Gaxiola, A. & Marquet, P.A. (2013) What drives plant species diversity across ecosystem development?. *In press*.

Anderson, D.W. (1988) The effect of parent material and soil development on nutrient cycling in temperate ecosystems. *Biogeochemistry*, **5**, 71-97.

Bååth, E. & Anderson, T.H. (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*,**35,** 955–963.

Bardgett, R.D., Bowman, W.D., Kaufmann, R. & Schmidt, S.K. (2005) A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology & Evolution*,**20,** 634–41.

Berendse, F. (1998) Effects of dominat plant species on soil during succession in nutrient-poor ecosystems. *Biogeochemistry*, **42**, 73-88.

Chadwick, O.A. & Chorover, J. (2001) The chemistry of pedogenic thresholds. *Geoderma*, **100**, 321–353.

Clarke, K.R. & Gorley, R.N. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Coomes, D.A., Allen, R.B., Bentley, W.A., Burrows, L.E., Canham, C.D., Fagan, L.*et al.* (2005) The hare, the tortoise and the crocodile: the ecology of angiosperm dominance, conifer persistence and fern filtering. *Journal of Ecology*, **93**, 918-935.

Cornelissen, J., Aerts, R., Cerabolini, B., Wergerm M. & Van Der Heijden, M. (2001). Carbon cycling traits of plant species are linked with mycorrhizal strategy. *Oecologia*,**129,** 611-619.

Crews, T.E., Kitayama, K., Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller-Dombois, D. and Vitousek, P.M. (1995) Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology*, **76**, 1407-1424.

Deubel, A. & Merbach, W. (2005) Influence of Microorganisms on Phosphorus Bioavailability in Soils. In: F. Buscot and A. Varma (eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag, Berlin Heidelberg, Germany.

Einen, J., Thorseth, I.H. & Ovreås, L. (2008) Enumeration of Archaea and Bacteria in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. *FEMS microbiology letters*,**282,** 182–7.

Eskelinen, A., Stark, S. & Männistö, M. (2009) Links between plant community composition, soil organic matter quality and microbial communities in contrasting tundra habitats. *Oecologia*,**161,** 113–23.

Esperschütz, J., Perez de Mora, A., Schreiner, K., Welzl, G., Buegger, F., Zeyer, J. *et al.* (2011) Microbial food web dynamics along a soil chronosequence of a glacier forefield. *Biogeosciences*,**8,** 3283–3294.

Fierer, N. & Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*,**103,** 626–31.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., & Cleveland, C.C. (2009) Global patterns in belowground communities. *Ecology Letters*, **12**, 1238-1249.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology*,**2,** 113-118.

Göransson, H., Olde Venterink, H. & Bååth, E. (2011) Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield. *Soil Biology and Biochemistry*,**43,** 1333–1340.

Green, J. & Bohannan, B.J.M. (2006) Spatial scaling of microbial biodiversity.*Trends in Ecology & Evolution*,**21,** 501–7.

Güsewell, S. & Gessner, M.O. (2009) N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. *Functional Ecology*, **23**, 211–219.

Huggett, R. (1998) Soil chronosequences, soil development, and soil evolution: a critical review. *Catena*,**32,** 155–172.

Jangid, K., Whitman, W.B., Condron, L.M., Turner, B.L.& Williams, M.A. (2013) Progressive and retrogressive ecosystem development coincide with soil bacterial community change in a dune system under lowland temperate rainforest in New Zealand. *Plant and Soil*,**367,** 234-247.

Kardol, P., Cornips, N.J., van Kempen, M.M., Bakx-Schotman, J.T. and Van Der Putten, W.H. (2007) Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*,**77,** 147–162.

Kemmitt, S., Wright, D., Goulding, K. & Jones, D. (2006) pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biology and Biochemistry*,**38,** 898–911.

Kuramae, E., Gamper, H., Van Veen, J. & Kowalchuk, G. (2011) Soil and plant factors driving the community of soil-borne microorganisms across chronosequences of secondary succession of chalk grasslands with a neutral pH. *FEMS microbiology ecology*,**77,** 285–94.

Laliberte, E., Turner, B.L., Costes, T., Pearse, S.J., Wyrwoll, K.H., Zemunik, G. *et al.* (2012) Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. *Journal of Ecology*,**100**, 631-642.

Lambers, H., Raven, J.A., Shaver, G.R. & Smith, S.E. (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*,**23,** 95–103.

Lane, D.J., Pace, B., Olsen,G.J., Stahl, D.A., Sogin, M.L. & Pace, N.R. (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*, **82**, 6955–6959.

Lauber, C.L., Strickland, M.S., Bradford, M.A. & Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry*, **40**, 2407–2415.

Legendre, P.&Gallagher, E. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia*,**129,** 271–280.

Lichter, J. (1998) Rates of weathering and chemical depletion in soils across a chronosequence of Lake Michigan sand dunes. *Geoderma*,**85,** 255–282.

Marschner, P., Yang, C., Lieberei, R. & Crowley, D.E. (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biology and Biochemistry*, **33**, 1437–1445.

Moore, J., Macalady, J.L., Schulz, M.S., White, A.F. & Brantley, S.L. (2010) Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. *Soil Biology and Biochemistry*, **42**, 21–31.

Peltzer, D.A., Wardle, D.A., Allison, V.J., Baisden, W.T., Bardgett, R.D., Chadwick, O.A. *et al.* (2010) Understanding ecosystem retrogression. *Ecological Monographs*, **80**, 509-529.

Placzek, C., Quade, J., Betancourt, J.L., Patchett, J.P., Rech, J.A., Latorre. C. *et al.*(2009) Climate in the Dry Central Andes over Geologic, Millennial, and Interannual Timescales.*Annals of the Missouri Botanical Garden*, **96,** 386–397.

Prévost-BouréN., Christen, R., Dequiedt, S., Mougel, C., Lelievre, M., Jolivet, C. *et al*. (2011) Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR. *PloS ONE*, **6**, e24166.

Rees, G.N., Baldwin, D.S., Watson, G.O., Perryman, S. & Nielsen, D.L. (2004) Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. *Antonie Van Leeuwenhoek*,**86,** 339–347.

Richardson, S.J., Peltzer, D.A., Allen, R.B., McGlone, M.S. & Parfitt, R.L. (2004) Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia*, **139**, 267–76.

Rousk, J., Brookes, P.C. & Bååth, E. (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and environmental microbiology*,**75,** 1589–96.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G.*et al.* (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME journal*,**4,** 1340–51.

Schmidt, S.K., Reed, S.C., Nemergut, D.R., Grandy, A.S., Cleveland, C.C., Weintraub, M.N. *et al.* (2008) The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. *Proceedings of Biological Science of The Royal Society*,**275,** 2793–802.

Schulz, S., Brankatschk, R., Dümig, A., Kögel-Knabner, I., Schloter, M.& Zeyer, J. (2013) The role of microorganism at different stages of ecosystem development for soil formation. *Biogeosciences*, **10**, 3983-3996.

Servicio Nacional de Geología y Minería (SGM). 1995 Carta geológica de Bolivia. Hoja Salinas de Garci Mendoza. Escala 1:250.000. Serie II-MTB-4B.

Servicio Nacional de Geología y Minería (SGM). 2002 Carta geológica de Bolivia. Hoja Villa Martin. Escala 1:250.000. Serie II-MTB-13B.

Strickland, M.S. & Rousk, J. (2010) Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications.*Soil Biology and Biochemistry*,**42,** 1385–1395.

Tarlera, S., Jangid, K., Ivester, A.H., Whitman, W.B. & Williams, M.A. (2008) Microbial community succession and bacterial diversity in soils during 77,000 years of ecosystem development. *FEMS microbiology ecology*,**64,** 129–40.

Tibaldi, A., Corzzato, C. & Rovida, A. (2009) Miocene-Quaternary structural evolution of the Uyuni-Atacama region, Andes de Chile and Bolivia. *Tectonophysics*,**471,** 114-135.

Ulrich, A. & Becker, R. (2006) Soil parent material is a key determinant of the bacterial community structure in arable soils. *FEMS microbiology ecology*,**56,** 430–43.

Urcelay, C., Acho, J. & Joffre, R. (2011) Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation.*Mycorrhiza*,**21,** 323–30.

Van Der Heijden, M.G., Bardgett, R.D. & Van Straalen, N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.

Van Der Wal, A., Van Veen, J.A., Smant, W., Boschker, T.S., Bloem, J., Kardol, P. *et al.* (2006) Fungal biomass development in a chronosequence of land abandonment. *Soil Biology and Biochemistry*, **38**, 51–60.

Vitousek, P.M. (2004) Nutrient Cycling and Limitation: Hawaii as a Model System. Princeton University Press.

Vuille, M., Bradley, R.S. & Keimig, F. (2000) Interannual climate variability in the Central Andes and its relation to tropical Pacific and Atlantic forcing.*Journal of Geophysical Research*,**105**, 12447-12460.

Wagai, R., Kitayama, K., Satomura, T., Fujinuma, R. & Balser, T. (2011) Interactive influences of climate and parent material on soil microbial community structure in Bornean tropical forest ecosystems. *Ecological Research*,**26,** 627–636.

Walker, T.W. & Syers, J.K. (1976) The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–19.

Walker, L.R. &del Moral, R. (2003) Primary succession and ecosystem rehabilitation. Cambridge University Press, Cambridge, UK.

Wardle, D.A., Bardgett, R.D., Walker, L.R., Peltzer, D.A. & Lagerström, A. (2008) The response of plant diversity to ecosystem retrogression: evidence from contrasting long-term chronosequences. *Oikos*, **117**, 93–103.

Wardle, D.A., Walker, L.R. & Bardgett, R.D. (2004a) Ecosystem properties and forest decline in contrasting long-term chronosequences.*Science*,**305,** 509-513.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H. & Wall D.H. (2004b) Ecological linkages between aboveground and belowground biota.*Science*,**304,** 1629–33.

Wardle, D.A., Yeates, G.W., Williamson, W. & Bonner, K.I. (2003) The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. *Oikos*,**102**, 45–56.

Welc, M., Bünemann, E.K., Fließbach, A., Frossard, E. & Jansa, J. (2012) Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. *Soil Biology and Biochemistry*,**49,** 184–192.

White, D. 2000. The Physiology and Biochemistry of Prokaryotes. Oxford University Press, Inc, New York.

Whitman, W.B., Coleman, D.C. & Wiebe W.J. (1998) Perspective Prokaryotes : The unseen majority. *Proceedings of the National Academy of Sciences*, **95**, 6578–6583.

Williams, M.A., Jangid, K., Shanmugam, S.G. & Whitman, W.B.(2013) Bacterial communities in soil mimic patterns of vegetative succession and ecosystem climax but are resilient to change between seasons. *Soil Biology and Biochemistry*,**57**, 749-757.

Williamson, W.M., Wardle, D.A.&Yeates, G.W. (2005) Changes in soil microbial and

nematode communities during ecosystem decline across a long-term chronosequence. *Soil Biology and Biochemistry*,**37,** 1289–1301.

Yavitt, J.B. (2000) Nutrient dynamics of soil derived from different parent material on Barro Colorado Island, Panama. *Biotropica*,**32**, 198-207.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. & Tilman, D. (2003) Plant diversity, soil microbial communities, and ecosystem function: are there any links?. *Ecology*,**84**: 2042–2050.

Zinger, L., Lejon, D.P., Baptist, F., Bouasria, A., Aubert, S., Geremia, R.A. *et al.*(2011) Contrasting diversity patterns of crenarchaeal, bacterial and fungal soil communities in an alpine landscape.*PloS ONE***6**: e19950.

Table1. Site characteristics across soil development in three chronosequences from Andean Dry Puna, Bolivia. Mean ± SE (Standard errors in parentheses) are given for each soil and plant characteristics (Modified from Alfaro et al, 2013).

www	Chronosequence			
	Sedimentary	Volcanic-1	Volcanic-2	*p*
Abiotic				
Position	19°58 S; 68°11 W	19°46 S; 67°34 W	20°21 S; 67°54 W	
Elevation (m.a.s.l.)	3834	3878	3866	
Slope (%)	47·96 (19·0)	48·01 (18·7)	33·46 (21·3)	
% C Total	**1·16** (0·17)↓	0·91 (0·10)	1·31 (0·16)	0·07
% N Total	**0·03** (0·005) ↑	**0·06** (0·01) ↑	**0·08** (0·01) ↑	**
P Total (mg kg^{-1})	**618·01** (21·82) ↓	783·83 (27·24)	779·30 (67·64)	**
P_{OLSEN} (mg kg^{-1})	12.86 (0·63)	18·33 (1·60)	**14·83** (0·85)	**
C:N(molar)	**90·77** (26·16) ↓	24·33 (8·59)	**55·27** (34·96)	0·12
N:P (molar)	**1·21** (0·22) ↑	**1·90** (0·29) ↑	**2·55** (0·33) ↑	**
pH	**9·02** (0·07)	**8·33** (0·19) ↓	**7·93** (0·25) ↓	**
Soil moisture£	0·33 (0·01)	0·29 (0·01)	0·23 (0·008) ↓	**
Salinity (dS m^{-1})	1·51 (0·30) ↓	1·01 (0·16) ↓	1·42 (0·32)	0·13
Biotic				
Plant richness	15·3 (0·59)	**20·5** (0·90) ↑	**20·6** (0·88) ↑	**
Plant cover (%)	**23·12** (2·15) ↓	**24·43** (1·49) ↓	**23·37** (2·10) ↓	0·69
Plant litter C:N	**78·97** (5·33) ↓	**63·41** (4·46) ↓	63·38 (2·76)	**

Data of parent material from SGM (1995, 2002), Tibaldi et al, 2009. £= (gr H_2O/gr soil).Values with arrows indicate statistical significant difference from one-way ANOVAs at p< 0·05 and trend (↑= increase; ↓=decline; and space = no tendency) across stages of soil development for individual chronosequences. *p* values indicate statistical significant difference from GLM Nested design ANOVA with stage nested in chronosequence (* p < 0.05; ** p < 0.01).

Table 2. Mean ± SE of fungal:bacterial ratio (gene copies g^{-1} soil) across stages of soil development in three chronosequences from Andean Dry Puna.

Biomass	Chronosequence type		
	Sedimentary	Volcanic-1	Volcanic-2
Bacteria	**5·92E+07** ($1{\cdot}3x10^7$)	**3·72E+07** ($5{\cdot}2x10^6$)	**5·88E+07** ($1{\cdot}1x10^7$)
Fungal	**6·24E+06** ($9{\cdot}3x10^5$)	**1·08E+07** ($9{\cdot}5x10^5$)	**1·07E+07** ($2{\cdot}0x10^6$)
Fungal:bacterial	**0·17** $(0{\cdot}04)^{a}$	**0·35** $(0{\cdot}04)^{b}$	**0·18** $(0{\cdot}02)^{a}$

Note: values in bold indicate statistical significant difference from one way ANOVAs at $p< 0{\cdot}05$ across ecosystem development. Letters on superscript on fungal:bacterial ratio mean indicate pairwise differences (Tukey HSD test) from and one way ANOVA at $p< 0{\cdot}05$ among chronosequences.

Table 3. Mantel test's correlation coefficient between microbial biomass (fungal:bacterial ratio) and environmental variables across soil development in three types of chronosequences with soils derived from distinct parent material.

Factors	Chronosequence type		
	Sedimentary	Volcanic-1	Volcanic-2
% C Total	**0·40**	0·02	0·12
% N Total	-0·03	0·01	-0·11
% P Total	**0·29**	0·08	0·09
P_{OLSEN}	0·06	-0·15	-0·12
C:N (molar)	0·14	-0·09	-0·14
N:P (molar)	-0·03	-0·03	0·04
pH	-0·10	0·15	0·01
Soil moisture (gr H_2O/gr soil)	0·31	**0·25**	0·02
Salinity	0·28	0·29	-0·09
PCC	0·09	0·09	0·21
Plant richness	0·22	0·22	0·07
Plant cover (%)	0·02	0·09	-0·01
Plant litter C:N	0·06	**0·26**	-0·17

Note: Values in bold indicate statistical significance at $P< 0·05$, after 5,000 permutations.

Table 4. Pearson's correlation coefficient between the first axis score of the NMDS ordination of microbial community composition and environmental variables, across three types of long-term chronosequences with soils derived from distinct parent material.

Factors	Bacteria			Fungi		
	Sedimentary	Volcanic-1	Volcanic-2	Sedimentary	Volcanic-1	Volcanic-2
% C Total	-0·05	0·34	-0·20	-0·03	0·03	-0·01
% N Total	-0·06	0·38	**0·49**	0·17	-0·10	0·13
% P Total	0·23	-0·22	-0·01	-0·19	-0·04	-0·02
P_{OLSEN}	0·44	-0·16	**-0·51**	0·14	-0·31	-0·03
C:N (molar)	-0·01	-0·22	-0·32	-0·02	-0·10	-0·47
N:P (molar)	-0·07	0·45	0·33	0·19	-0·07	0·09
pH	-0·01	**-0·72**	-0·38	0·27	-0·14	**-0·55**
Soil moisture	-0·24	-0·29	**-0·53**	-0·20	-0·41	-0·32
Salinity	-0·16	-0·47	**-0·51**	0·15	**-0·58**	-0·45
PCC	0·17	**0·70**	**0·54**	0·14	0·35	0·15
Plant richness	**-0·54**	**0·58**	0·41	-0·06	0·43	0·33
Plant cover (%)	-0·46	-0·22	-0·46	-0·31	-0·13	**-0·62**
Plant litter C:N	0·18	**-0·68**	0·22	0·06	**-0·49**	-0·03

Note: values in bold indicate statistical significance at $p < 0·05$.

Figure legends

Figure 1. Site location, **(a)** Map of the Central Andean Dry Puna, Bolivia. The shaded area around the Uyuni salt flat (white area) indicates the surface flooded during the Tauca cycle (gray) and the Coipasa cycle (light gray). **(b)** The chronosequences system in the Uyuni salt flat. Gray scale circles indicate the chronosequences locations and parent material type; and **(c)** Schematic representation of the chronosequence formation by flood disturbance during the past 18,000 years. On the left the maximum elevation (mts) reached by water during the flood events, and on the right the time elapsed since each event. USF indicates the actual elevation (m.a.s.l.) of the Uyuni salt flat.

Figure 2. Biomass measure (fungal and bacterial gene copies g^{-1} soil) expressed in fungal:bacterial ratio among **(a)** chronosequences (sedimentary, volcanic-1 and volcanic-2) and **(b-d)** stages of soil development for the three types of chronosequences. Data shown are means ± s.e.

Figure 3. Microbial community composition (based on the T-RFLP method) among chronosequences and stages of soil development, Non-metric multidimensional scaling ordination (NMDS) analyses of bacterial **(a)** and fungal **(b)** community composition among type of chronosequence. NMDS analyses of bacterial and fungal community composition across soil development in sedimentary **(c** and **d)**, volcanic-1 **(e** and **f)** and volcanic-2 **(g** and **h)** chronosequences. Pairwise comparisons using the Bonferroni correction to maintain $\alpha = 0{\cdot}05$. Values obtained for the two ordination axes were used to estimate centroids for each chronosequence and stage. The R and p values obtained from one-way ANOSIM represent the dissimilarity among communities and the significance respectively.

Figure 4. Pearson correlation between fungal:bacterial ratio and decomposition (Whatman filter papers) across soil development in **(a)**sedimentary, **(c)** volcanic-1 and **(e)** volcanic-2 chronosequences. Spearman correlation between soil microbial (bacterial and fungal) abundance (gene copies g^{-1} soil) and decomposition across soil development in **(b)** sedimentary, **(d)** volcanic-1 and **(f)** volcanic-2 chronosequences. (open circle: bacteria; filled circle: fungi).

Figure 5. Bray-Curtis dissimilarity of microbial communities plotted against geographic distance, pH-distance and plant community composition for all pairwise comparisons **(a-c)** bacterial dissimilarity and **(d-f)** fungi dissimilarity across soil development for the three types of chronosequences (on top values of coefficient of determination and significance).

Figure S1. Effect of soil pH and plant community composition on BCC and FCC changes across oil development in volcanic-1 and volcanic-2 chronosequences.**(a-b)**Non-metric multidimensional scaling ordination (NMDS) of bacterial communities from volcanic-1 and volcanic-2 chronosequences.**(c-d)** Cluster of BCC grouped by soil pH category and plant community composition. **(e-f)** Change in soil pH and plant community composition across soil development in volcanic-1 and volcanic-2 chronosequences.

Figure 1.

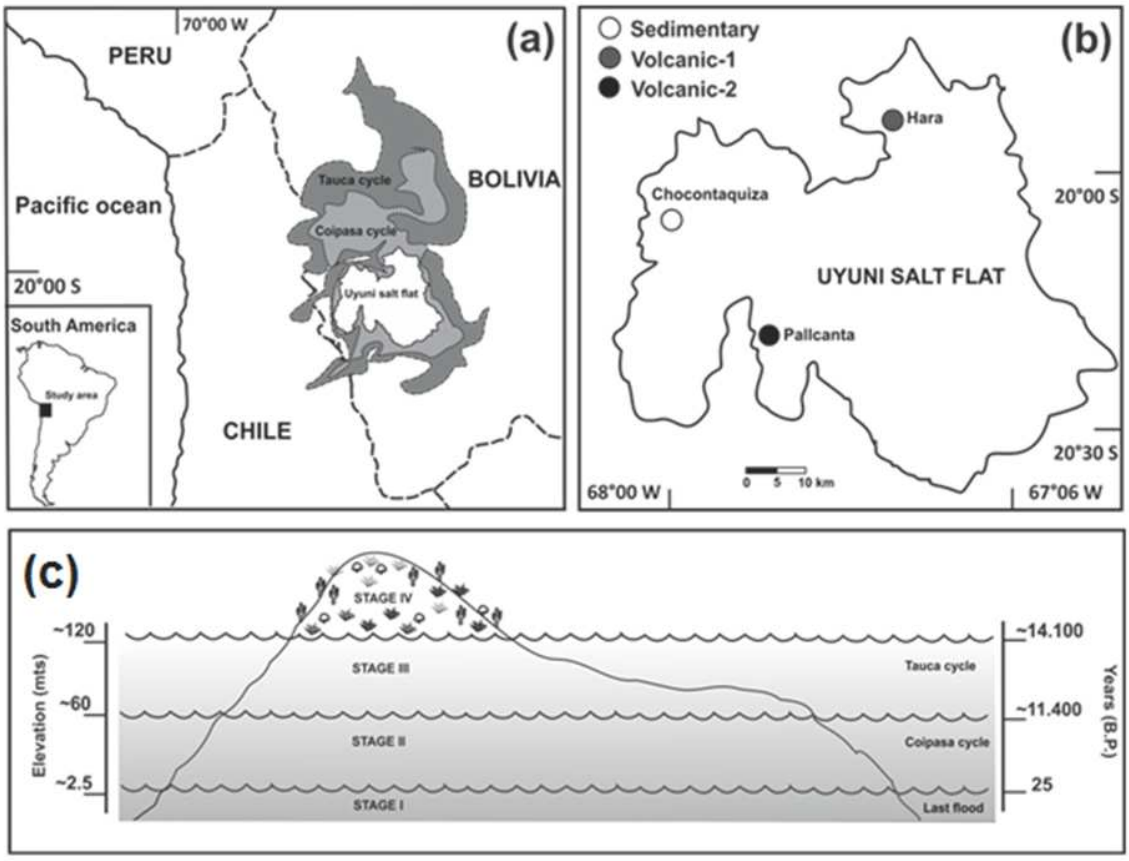

(a)
70°00 W
PERU
BOLIVIA
CHILE
Pacific ocean
20°00 S
South America
Study area
Tauca cycle
Coipasa cycle
Uyuni salt flat
(b)
Sedimentary
Volcanic-1
Volcanic-2
Hara
Chocontaquiza
20°00 S
UYUNI SALT FLAT
Pallcanta
20°30 S
68°00 W
0 5 10 km
67°06 W
(c)
STAGE IV
STAGE III
STAGE II
STAGE I
~120
~60
~2.5
Elevation (mts)
~14.100
~11.400
25
Years (B.P.)
Tauca cycle
Coipasa cycle
Last flood

Figure 2.

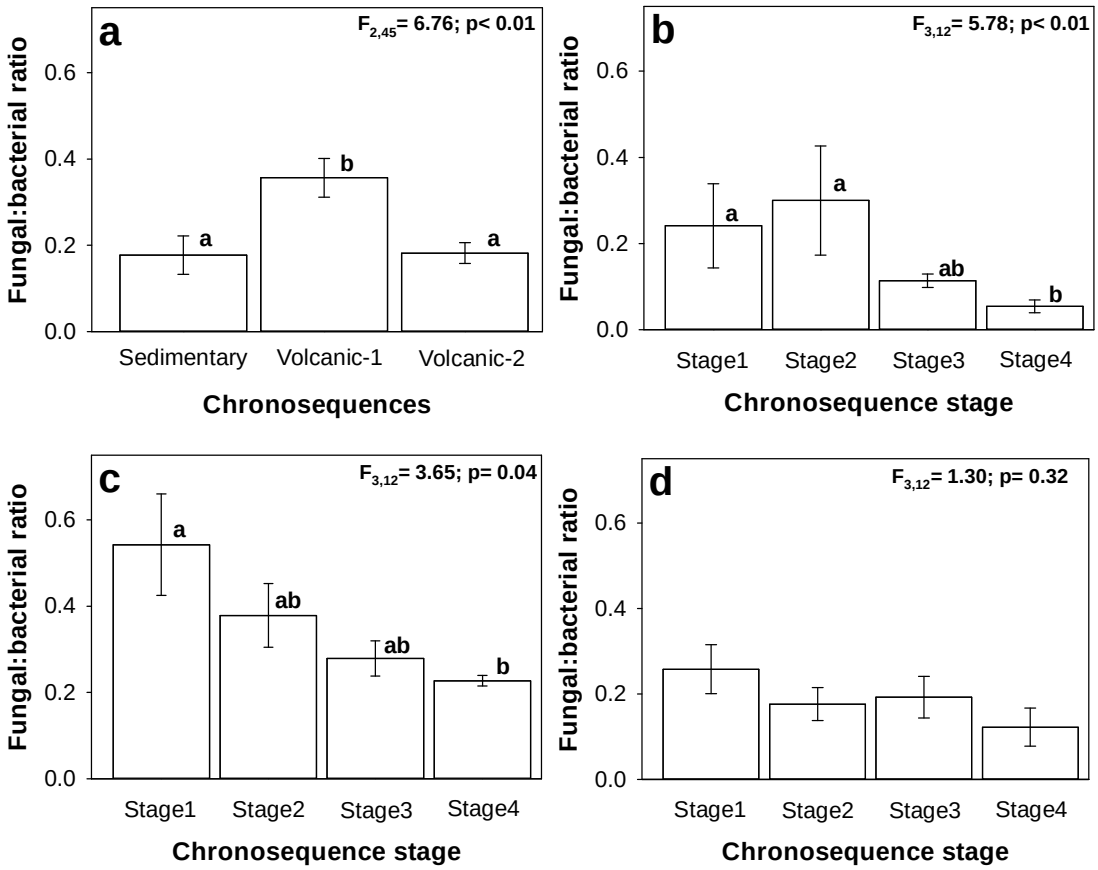

a
$F_{2,45}$= 6.76; p< 0.01
Fungal:bacterial ratio
0.0
0.2
0.4
0.6
a
b
a
Sedimentary
Volcanic-1
Volcanic-2
Chronosequences
b
$F_{3,12}$= 5.78; p< 0.01
a
a
ab
b
Stage1
Stage2
Stage3
Stage4
Chronosequence stage
c
$F_{3,12}$= 3.65; p= 0.04
a
ab
ab
b
Chronosequence stage
d
$F_{3,12}$= 1.30; p= 0.32
Chronosequence stage

Figure 3.

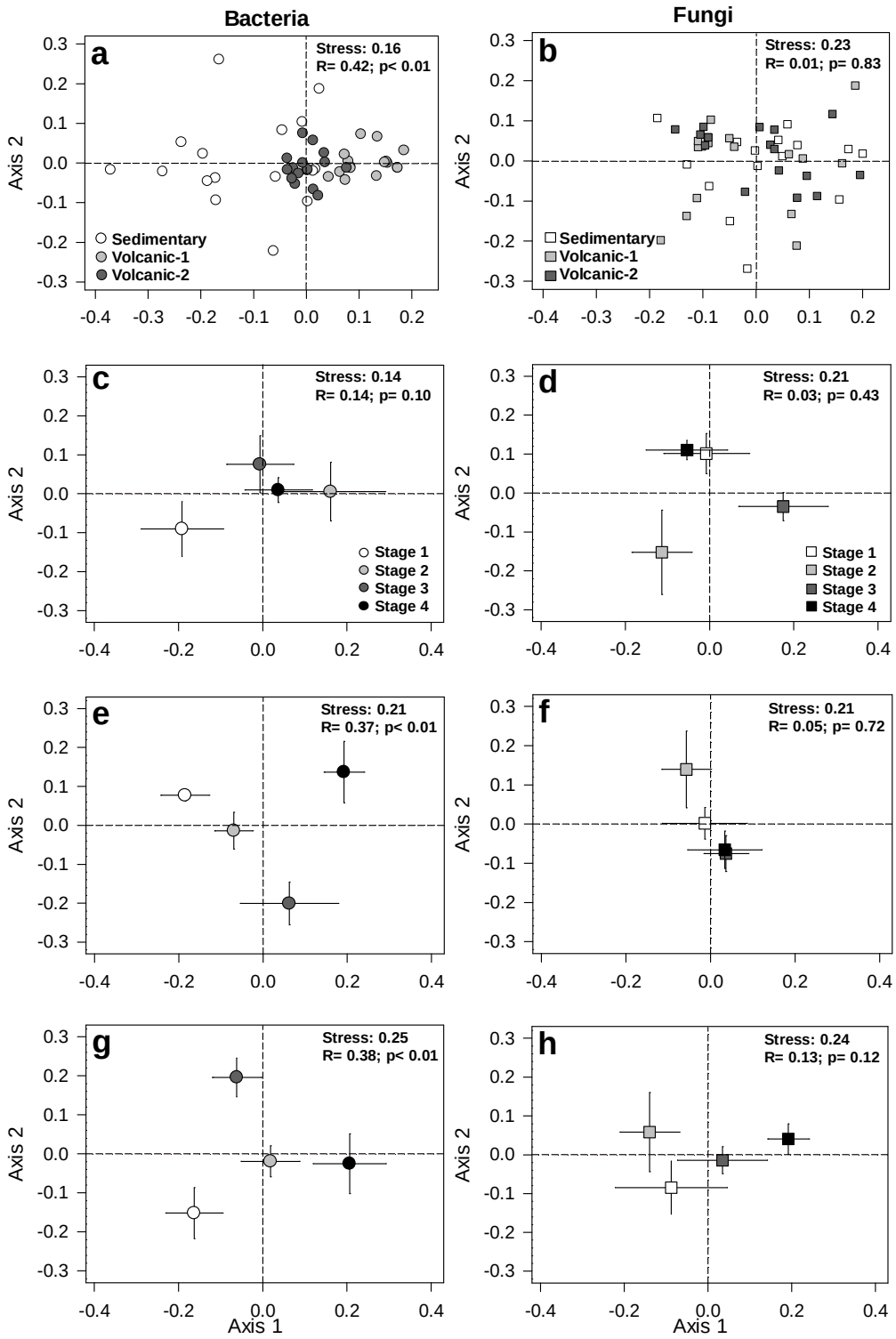

Bacteria
Fungi
a
Stress: 0.16
R= 0.42; p< 0.01
Sedimentary
Volcanic-1
Volcanic-2
b
Stress: 0.23
R= 0.01; p= 0.83
Sedimentary
Volcanic-1
Volcanic-2
c
Stress: 0.14
R= 0.14; p= 0.10
Stage 1
Stage 2
Stage 3
Stage 4
d
Stress: 0.21
R= 0.03; p= 0.43
Stage 1
Stage 2
Stage 3
Stage 4
e
Stress: 0.21
R= 0.37; p< 0.01
f
Stress: 0.21
R= 0.05; p= 0.72
g
Stress: 0.25
R= 0.38; p< 0.01
h
Stress: 0.24
R= 0.13; p= 0.12
Axis 1
Axis 2

Figure 4.

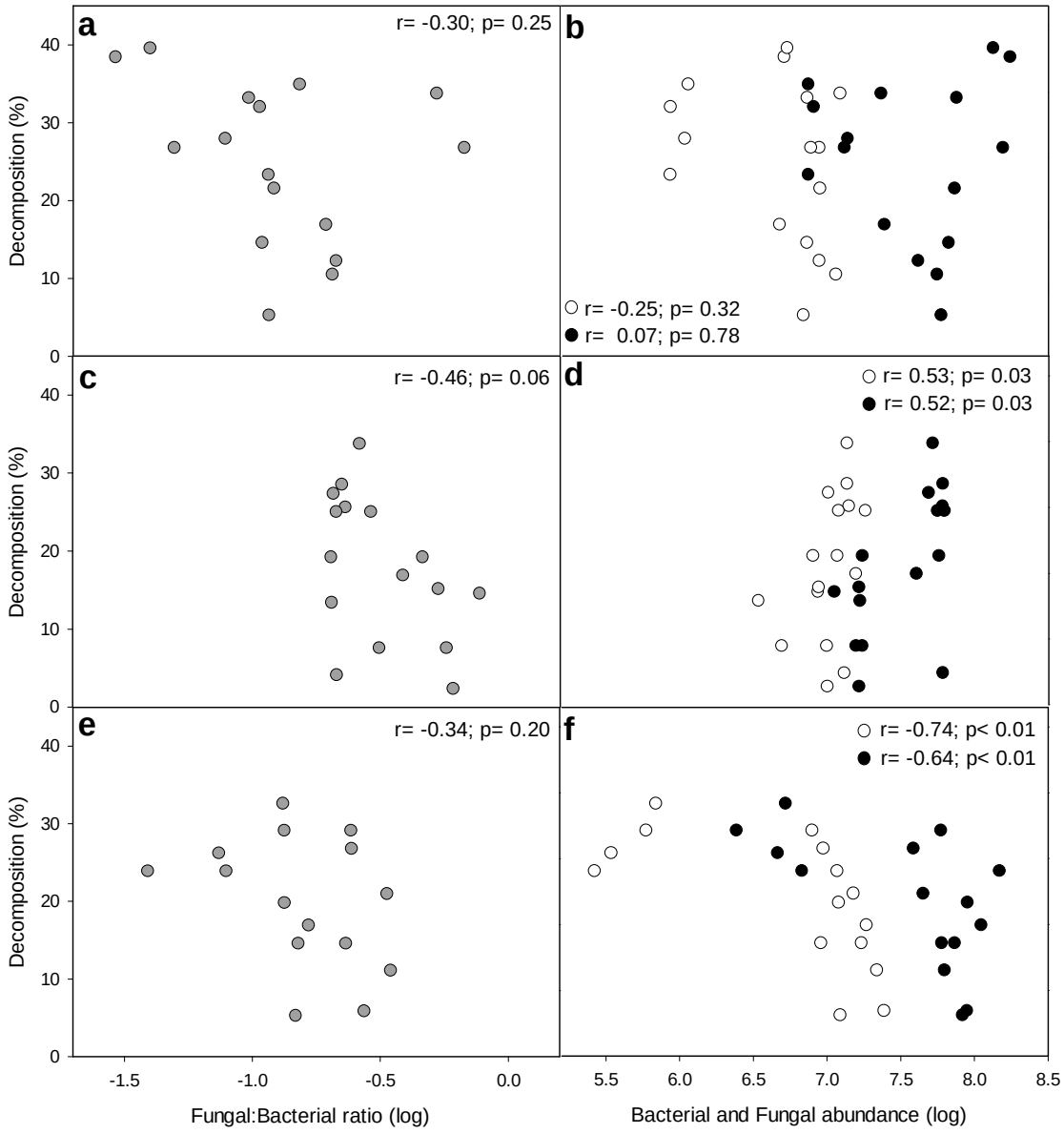
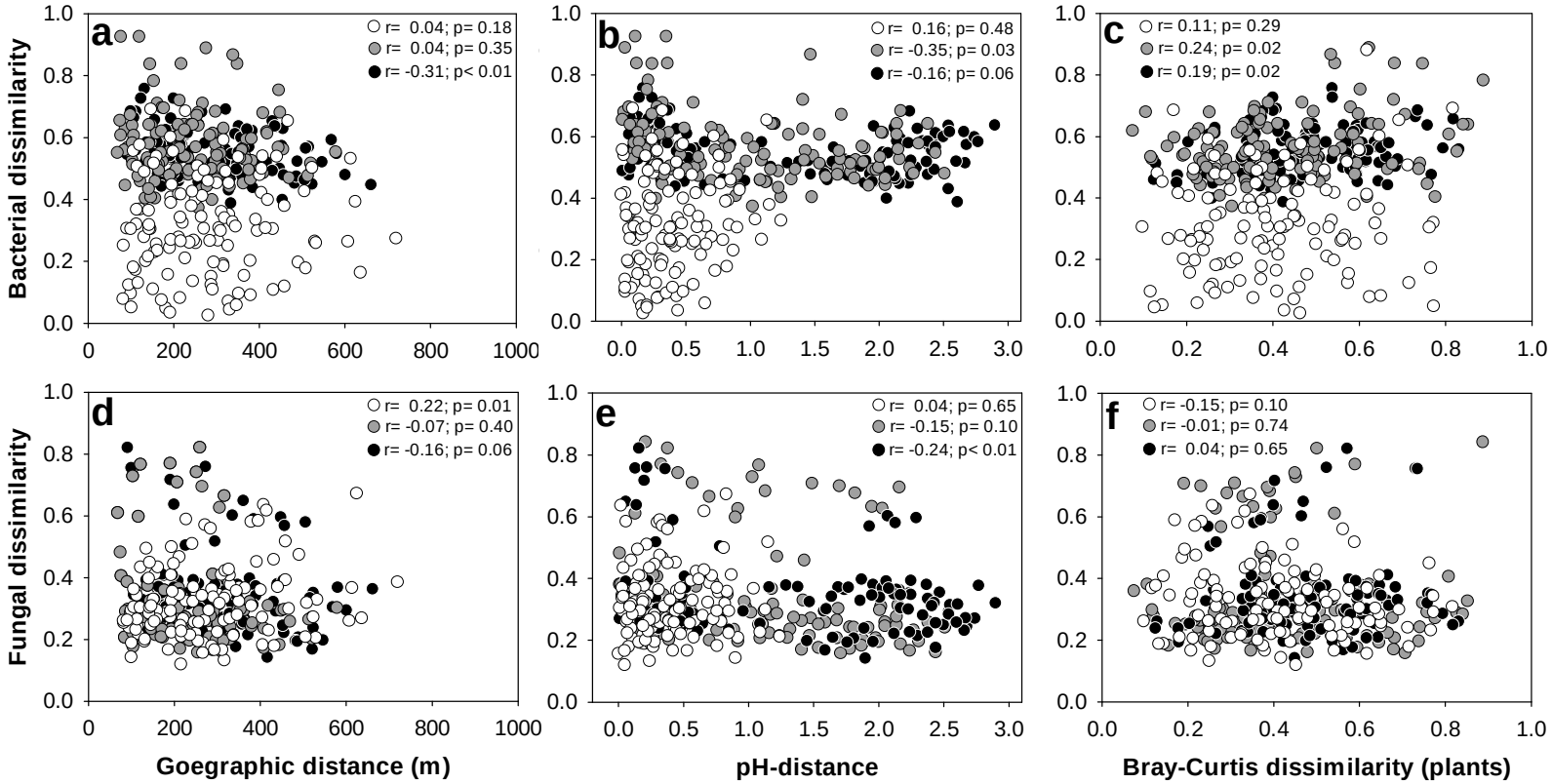

a
r= -0.30; p= 0.25
b
r= -0.25; p= 0.32
r= 0.07; p= 0.78
c
r= -0.46; p= 0.06
d
r= 0.53; p= 0.03
r= 0.52; p= 0.03
e
r= -0.34; p= 0.20
f
r= -0.74; p< 0.01
r= -0.64; p< 0.01
Decomposition (%)
Fungal:Bacterial ratio (log)
Bacterial and Fungal abundance (log)

Figure 5.


a
r= 0.04; p= 0.18
r= 0.04; p= 0.35
r= -0.31; p< 0.01
b
r= 0.16; p= 0.48
r= -0.35; p= 0.03
r= -0.16; p= 0.06
c
r= 0.11; p= 0.29
r= 0.24; p= 0.02
r= 0.19; p= 0.02
d
r= 0.22; p= 0.01
r= -0.07; p= 0.40
r= -0.16; p= 0.06
e
r= 0.04; p= 0.65
r= -0.15; p= 0.10
r= -0.24; p< 0.01
f
r= -0.15; p= 0.10
r= -0.01; p= 0.74
r= 0.04; p= 0.65
Bacterial dissimilarity
Fungal dissimilarity
Goegraphic distance (m)
pH-distance
Bray-Curtis dissimilarity (plants)

Supplementary material

Table S1. Pearson coefficient correlation between fungal and bacterial biomass (gene copies g^{-1} soil) and environmental variables across soil development.

Factors	Bacteria			Fungi		
	Sedimentary	Volcanic-1	Volcanic-2	Sedimentary	Volcanic-1	Volcanic-2
% C Total	-0·30	-0·12	0·08	**0·61**	-0·44	0·25
% N Total	0·20	-0·23	-0·06	**-0·58**	-0·39	-0·16
% P Total	-0·39	-0·38	-0·06	0·24	-0·19	0·25
P_{OLSEN}	-0·32	-0·16	**0·57**	0·04	-0·35	**0·47**
C:N (molar)	-0·16	0·27	0·01	**0·70**	0·13	-0·02
N:P (molar)	0·29	-0·13	0·07	**-0·54**	-0·32	-0·28
pH	**-0·83**	**-0·78**	**0·53**	-0·29	**-0·60**	**0·53**
Soil moisture	-0·34	**-0·60**	0·29	-0·23	0·39	0·37
Salinity	-0·24	**-0·50**	0·23	**0·62**	-0·24	0·23
PCC	0·02	**0·50**	-0·04	**-0·66**	0·28	0·41
Plant richness	0·15	0·41	0·08	0·17	0·41	-0·30
Plant cover (%)	**-0·53**	**-0·51**	**0·60**	-0·13	-0·40	**0·61**
Plant litter C:N	**-0·73**	**-0·67**	0·18	-0·30	-0·44	0·18

*Note:*Values in bold indicate statistical significance at P< 0·05.
PCC: First axis of RDA ordination of plant community composition.

Table S2. Values of R-similarity for one-way ANOSIM pairwise comparisons of bacterial (**Global R= 0·42**; p= 0·001) and fungal (**Global R=** 0·01; p= 0·83) community composition among different type of chronosequences.

Chronosequence	Parent material		
	Sedimentary	Volcanic-1	Volcanic-2
Sedimentary	-------	**0·55**	**0·24**
Volcanic-1	0·03	-------	**0·58**
Volcanic-2	0·03	0·01	-------

*Note:*R values in bold indicate statistical significance at P< 0·05.
Top right of the table: results from bacterial communities; and bottom left of the table: results from fungal communities.

Table S3. Results from one-way ANOSIM analyses testing for effects of chronosequence stage (S1 = youngest) on bacterial and fungal community composition.

Stage	Chronosequence		
	Sedimentary	Volcanic-1	Volcanic-2
Bacteria (Global R)	0·14	**0·37**	**0·38**
Stage pairwise comparisons			
S1 vs S2	0·38	0·03	0·25
S1 vs S3	0·13	**0·58**	**0·69**
S1 vs S4	0·35	**0·60**	**0·60**
S2 vs S3	0·02	0·30	0·36
S2 vs S4	0·01	0·44	0·04
S3 vs S4	0·09	**0·37**	**0·39**
Fungi (Global R)	0·09	0·05	0·13
Stage pairwise comparisons			
S1 vs S2	0·14	0·14	0·20
S1 vs S3	0·03	0·04	0·42
S1 vs S4	0·23	0·07	0·22
S2 vs S3	0·30	0·16	0·01
S2 vs S4	0·18	0·04	0·73
S3 vs S4	0·15	0·18	0·04

*Note:*R values near0 indicate no difference between groups, whereas values close to 1 indicate high dissimilarity between groups (in bold indicate statistical significance at P< 0·05). All *post-hoc* significance tests used the Bonferroni correction

Table S4. Among chronosequences correlation coefficient between first axis of NMDS ordination of microbial community composition and environmental variables; and simple Mantel test´s correlation coefficient between fungal:bacterial ratio change among chronosequences and environmental variables.

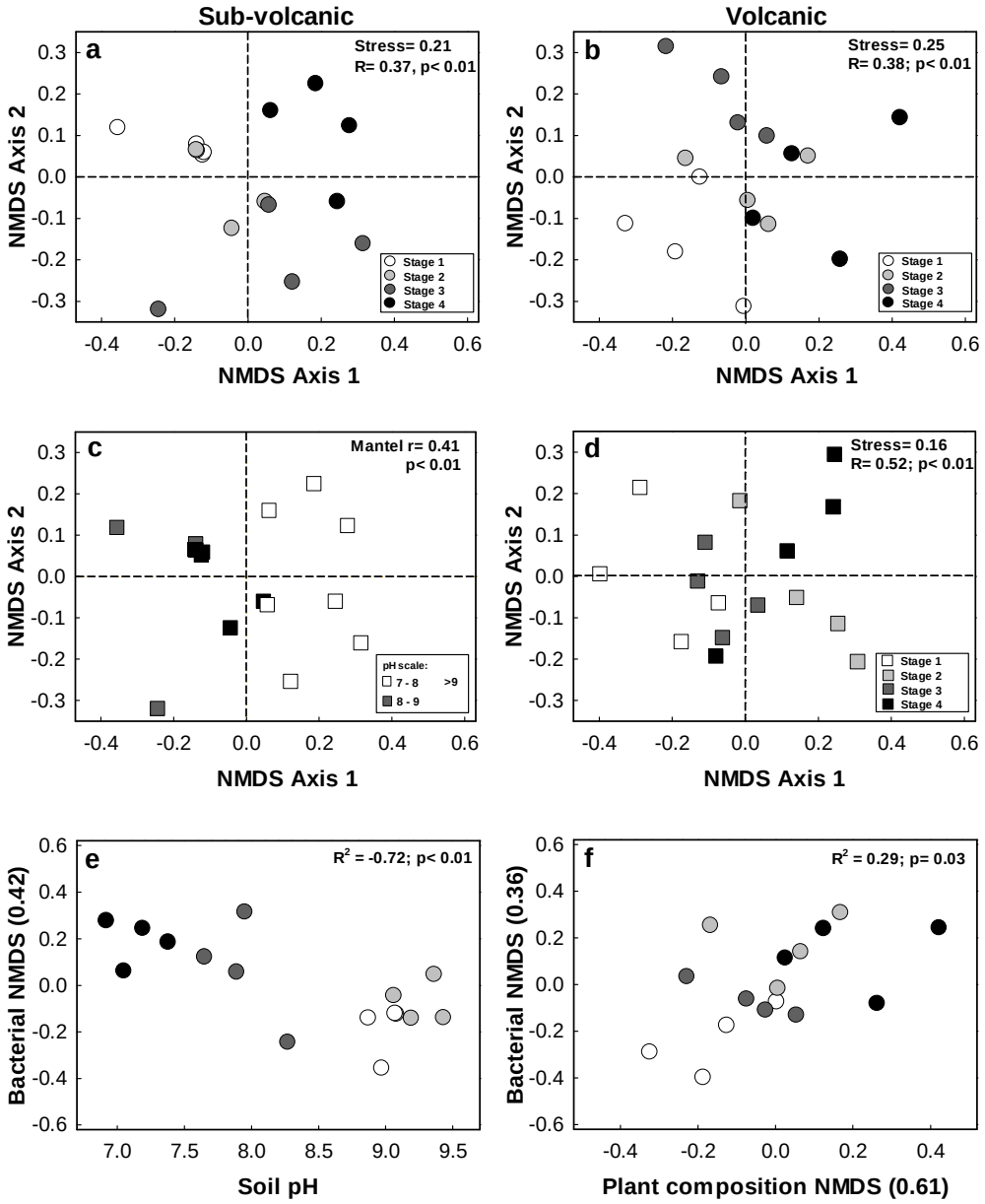
Factors	Microbial composition		Fungal:bacterial ratio
	Bacteria	Fungi	
Distance[1]	-	-	0·38
% C Total	-0·18	-0·01	0·39
% N Total	**0·28**	0·09	-0·57
% P Total	**0·29**	0·06	-0·72
P_{OLSEN}	**0·49**	-0·01	-0·75
C:N	-0·22	0·21	-0·89
N:P (molar)	0·16	-0·06	-0·15
pH	-0·21	**0·41**	-0·67
Soil moisture	-0·22	0·28	-0·57
Salinity	-0·14	0·24	-0·46
PCC	**0·30**	-0·04	-0·08
Plant richness	0·24	-0·20	-0·68
Plant cover (%)	0·11	**0·39**	-0·47
Plant litter C:N	-0·26	**-0·29**	-0·02

Note: Values in bold indicate statistical significance at P< 0·05, after 5,000 random permutations.
PCC: Plant community composition.
[1]= Represents a matrix of geographic distance (straight-line distance) between pairs of chronosequences.

Table S5. Values from standard curves of qPCR nucleic acids quantification and evaluation of analysis. Individual values are shown each chronosequence

Chronosequence	Standard curve qPCR							
	Bacteria				Fungi			
	Slope	Y-Intercept	Efficiency (%)	R^2	Slope	Y-Intercept	Efficiency (%)	R^2
Sedimentary	-3·219	28·19	104·50	0·98	-3·378	34·23	97·72	0·99
Volcanic-1	-3·217	27·73	104·53	0·99	-3·219	34·33	104·49	0·97
Volcanic-2	-3·172	27·99	106·65	0·99	-3·170	33·51	106·77	0·99

Figure S1.


Sub-volcanic
Volcanic
a
Stress= 0.21
R= 0.37, p< 0.01
b
Stress= 0.25
R= 0.38; p< 0.01
c
Mantel r= 0.41
p< 0.01
pH scale:
7 - 8
>9
8 - 9
d
Stress= 0.16
R= 0.52; p< 0.01
e
R² = -0.72; p< 0.01
f
R² = 0.29; p= 0.03
Stage 1
Stage 2
Stage 3
Stage 4
NMDS Axis 1
NMDS Axis 2
Bacterial NMDS (0.42)
Soil pH
Bacterial NMDS (0.36)
Plant composition NMDS (0.61)

CONCLUSIONES GENERALES

1. Desarrollo de los suelos y sus efectos sobre el funcionamiento y estructura de las comunidades de plantas y microorganismos del suelo

En esta tesis se presentaron los resultados de la primera investigación sobre el desarrollo a largo plazo del suelo en tres cronosecuencias edáficas que difieren en el origen del material parental, pero que han estado sujetas a las mismas condiciones climáticas y patrones de perturbación. Además se presentó evidencia sobre los efectos de la baja disponibilidad de N sobre el desarrollo de los suelos y sobre procesos ecosistémicos tales como la producción de biomasa, descomposición y el reciclaje de nutrientes, así como sobre los patrones de diversidad y composición de distintos grupos taxonómicos y funcionales de organismos que van desde las bacterias del suelo hasta las comunidades de plantas vasculares.

Contrario a las predicciones establecidas por modelos clásicos sobre el desarrollo de los suelos, el contenido de P se mantuvo sin cambios significativos a lo largo del desarrollo de los suelos en los tres tipos de cronosecuencias estudiadas. De esta manera, el incremento del N a lo largo del gradiente de edad de los suelos asociado a un reducido cambio en el contenido de P determinaron que la razón N:P incrementara durante las etapas tardías del desarrollo de los suelos en los tres tipos de cronosecuencias. Sin embargo, el incremento en la razón N:P durante el desarrollo de los suelos fue marcadamente menor a los valores registrados en cronosecuencias de rangos similares de edad situadas en ambientes mésicos (Fig. 1). Además, los valores de N:P de las cronosecuencias estudiadas presentaron valores por debajo del umbral de N:P = 16 que establece el cambio de la limitación por N a la limitación por P. Por lo tanto, los resultados aquí presentados sugieren que todas las etapas de desarrollo de las tres cronosecuencias se encuentran bajo una fuerte limitación por N.

Los valores de N, P y pH del suelo registrados en las últimas etapas después de 20,000 años de desarrollo de los suelos fueron marcadamente distintos a los observados en cronosecuencias de ambientes mésicos en donde la limitación por P se presenta en etapas tempranas del desarrollo de los suelos (Fig. 2).

Los cambios en las propiedades de los suelos a lo largo del gradiente de edad determinaron cambios en los procesos ecosistémicos tales como la producción de biomasa, descomposición y la reabsorción de los nutrientes. El cambio desde suelos altamente alcalinos, salobres y con bajo contenido de nutrientes (notablemente N) en etapas iniciales de desarrollo a suelos neutros, moderadamente salinos y con mayor contenido de nutrientes al final del gradiente de edad, determinaron una relación positiva entre las tasas de descomposición y la edad de suelos, además de una reducción en la reabsorción de nutrientes desde las hojas senescentes en plantas. La producción de biomasa presentó un patrón unimodal en relación con el gradiente de edad en los tres tipos de cronosecuencias (Fig. 3a), y este patrón fue asociado al incremento en la razón N:P y las disminuciones de pH y salinidad de los suelos.

Del mismo modo, el incremento en la razón N:P y la disminución en el pH junto con la salinidad de los suelos a lo largo de las cronosecuencias determinaron cambios en los patrones de plantas desde el nivel de rasgos intraespecíficos hasta patrones comunitarios tales como la riqueza de especies, composición y la intensidad y frecuencia de interacciones planta-planta. Es así, que el contenido de N:P en la hojarasca de las dos especies de plantas distribuidas a lo largo de todas las etapas de las tres cronosecuencias estudiadas incrementaron a medida que el N:P del suelo incrementó con la edad de los suelos. De igual manera, los cambios en la composición y el incremento en la riqueza de especies de plantas

durante el desarrollo de los suelos fueron determinados por los incrementos en las razones N:P de los suelos (Capítulo 2).Incluso, el cambio en las interacciones planta-planta a lo largo de las cronosecuencias fue mayormente determinado por cambios en la razón N:P y el otros factores del suelo tales como el pH y la salinidad.

Los patrones de abundancia y composición de las comunidades de bacterias y hongos del suelo a lo largo del desarrollo de las cronosecuencias se encuentran controlados por los cambios en las características de los suelos, tales como el contenido de nutrientes y las propiedades fisicoquímicas. Es así, que a medida que el pH y la salinidad declinaron con la edad de los suelos, se observó un incremento sostenido en la abundancia de bacterias; mientras que la disminución en la abundancia de hongos durante etapas tardías de desarrollo de los suelos fue mayormente determinado por cambios en el contenido de nutrientes en el suelo (notablemente N). De esta manera, la razón Hongo:Bacteria que expresa la dominancia microbiana, declinó durante estados tardíos del desarrollo de los suelos (Fig. 4), lo que sugiere un incremento en las tasas a las cuales ocurren procesos ecosistémicos tales como la descomposición y el reciclaje de nutrientes hacia el final del desarrollo de los suelos.

Las comunidades de bacterias presentaron un elevado recambio entre las etapas de desarrollo, lo que permitió diferenciar comunidades específicas en cada etapa de las cronosecuencias volcánicas-1 y volcánicas-2. Las comunidades de hongos, por otro lado, no variaron a lo largo del desarrollo en todos los tipos de cronosecuencias. El tipo de material parental de las cronosecuencias fue un factor clave que afectó el tipo de patrones de las comunidades de bacterias durante el desarrollo de los suelos, sin embargo, este efecto no se encontró en las comunidades de hongos. En conjunto, estos resultados son

consistentes con las observaciones que establecen un cambio en la dominancia microbiana asociado a la variación en las condiciones de los suelos, y resaltan el efecto del tipo de material parental en los patrones comunitarios de microorganismos del suelo, en particular sobre las comunidades de bacterias (Capítulo 3).

2. El efecto de material parental sobre el desarrollo de los suelos

Las diferencias observadas en el patrón de desarrollo de los suelos entre cronosecuencias con suelos derivados a partir de distinto material parental sugieren fuertemente que el origen geológico de los suelos tendría un efecto crítico sobre la pedogenesis de largo plazo y sobre los patrones y procesos ecosistémicos asociados. En particular, porque los tres tipos de cronosecuencias estudiadas presentaron patrones de diversidad y composición de especies de plantas y microorganismos muy distintos, además de grandes diferencias en el contenido y la reabsorción de nutrientes en las hojas y hojarasca de las plantas durante el desarrollo de los suelos. Todos estos resultados sugieren que tanto en la estructura como en el funcionamiento estos tres tipos de cronosecuencias fueron ampliamente distintos, independientemente de presentar el mismo rango de variación temporal en el desarrollo de los suelos y que experimentan similares condiciones climáticas. De esta manera, estos resultados llaman a incorporar explícitamente el efecto del tipo de material parental de los suelos en futuros trabajos sobre la pedogenesis y sus efectos sobre los componentes bióticos sobre y debajo del suelo.

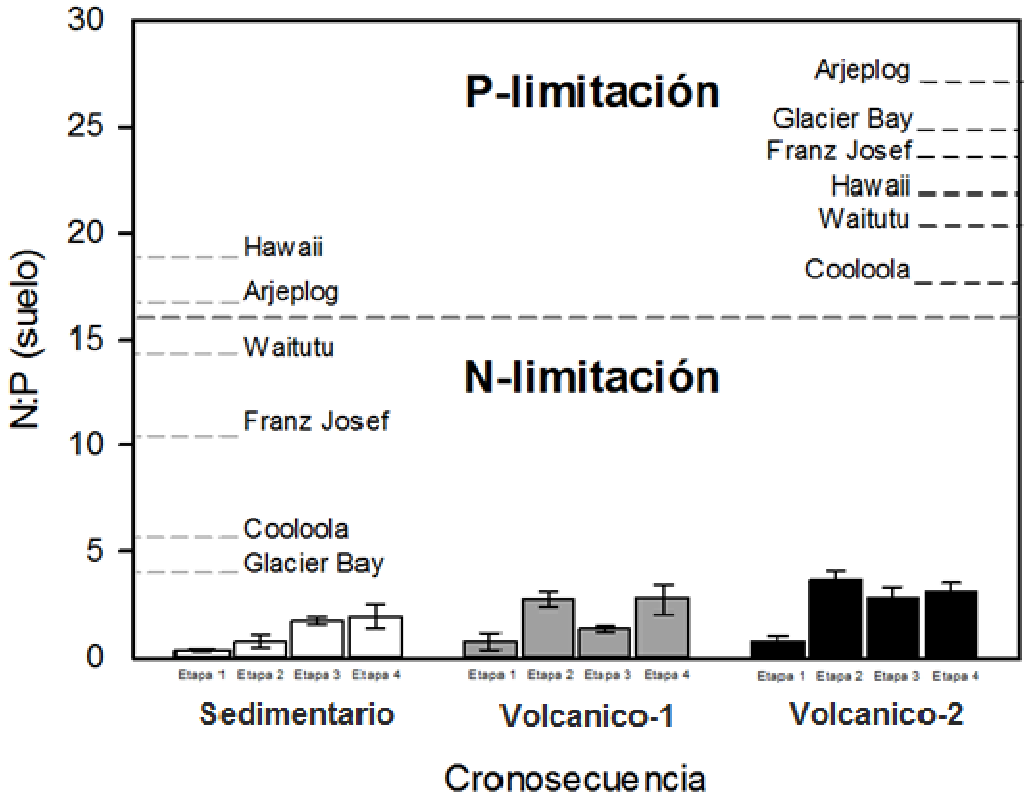


Figura 1. Comparación de la razón N:P del suelo de las cronosecuencias presentes en Uyuni-SF y las cronosecuencias reportadas en Wardle et al, (2004; 2008). La línea punteada representa el umbral de cambio de la limitación por N a una limitación por P. La posición de los nombres de las cronosecuencias en el eje de la izquierda indican los valores de las razón N:P en la primera etapa de desarrollo, mientras que la posición en el eje de la derecha indican los valores en las etapas cercana a los 20,000 años.

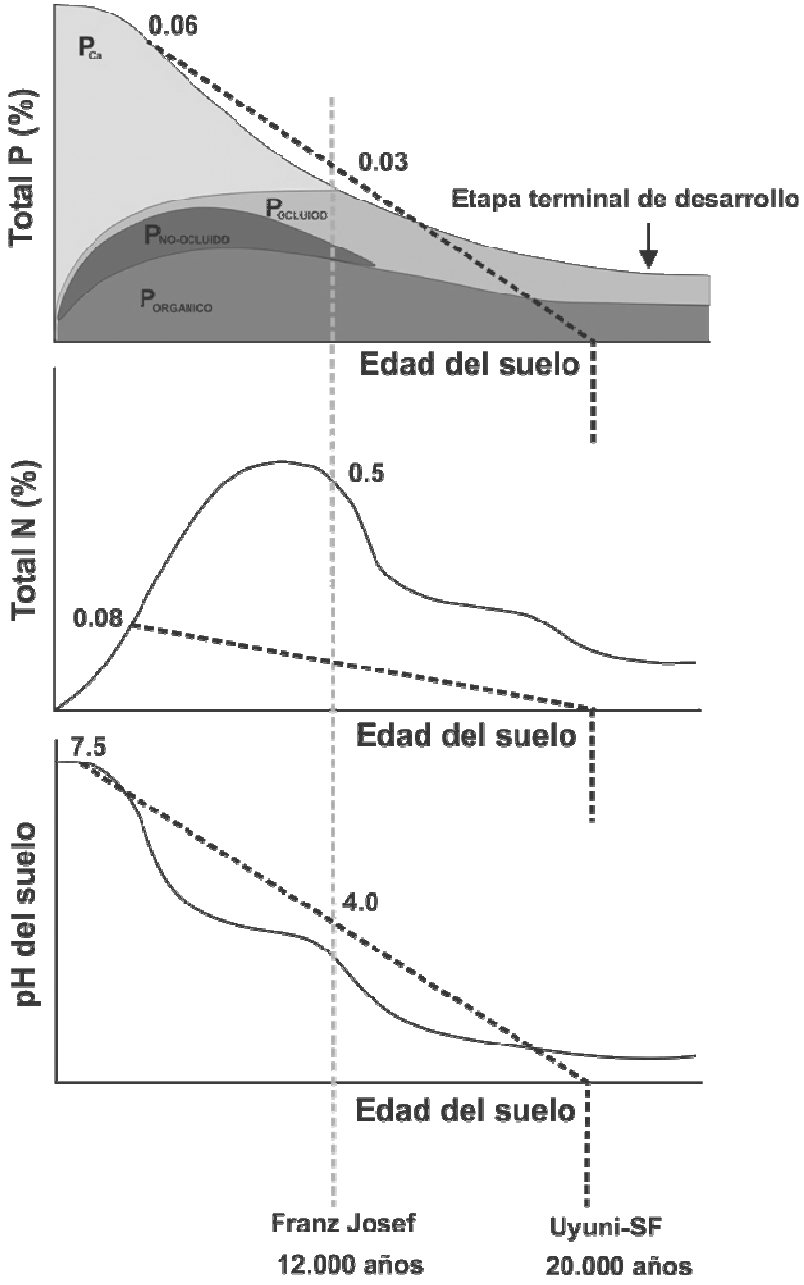


Figura 2. Comparación del contenido de N, P y el pH del suelo en la etapa final de las cronosecuencias de Uyuni-SF (valor promedio de los tres tipos de cronosecuencias) y la cronosecuencias de Franz Josef en Nueva Zelandia.

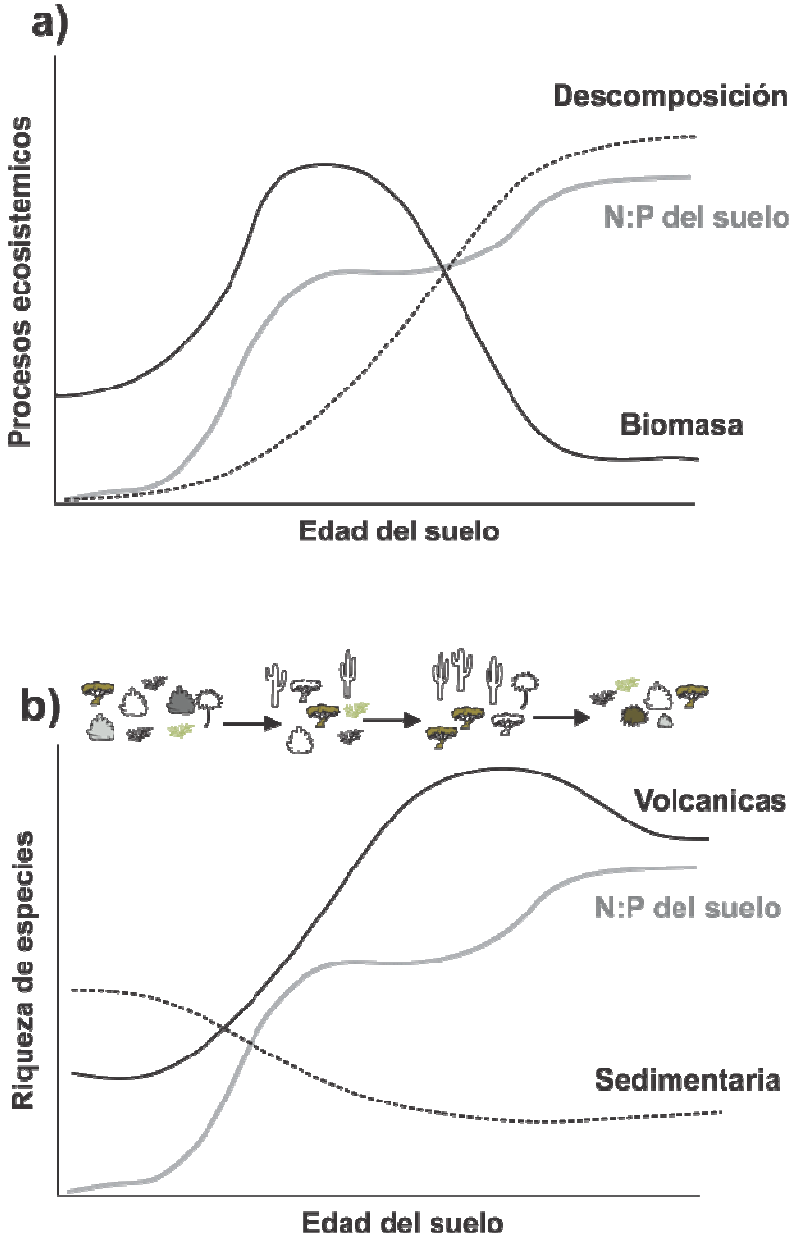


Figura 3. Cambios en los procesos ecosistémicos y patrones comunitarios a lo largo del desarrollo de los suelos en las cronosecuencias de Uyuni-SF. **a)** Incremento de la descomposición y disminución de la biomasa de plantas hacia el final del desarrollo de los suelos (valores promedio de las tres cronosecuencias). **b)** Cambio en la riqueza de especies de plantas y en la composición de especies de plantas a lo largo del desarrollo de los suelos en los tres tipos de cronosecuencias.

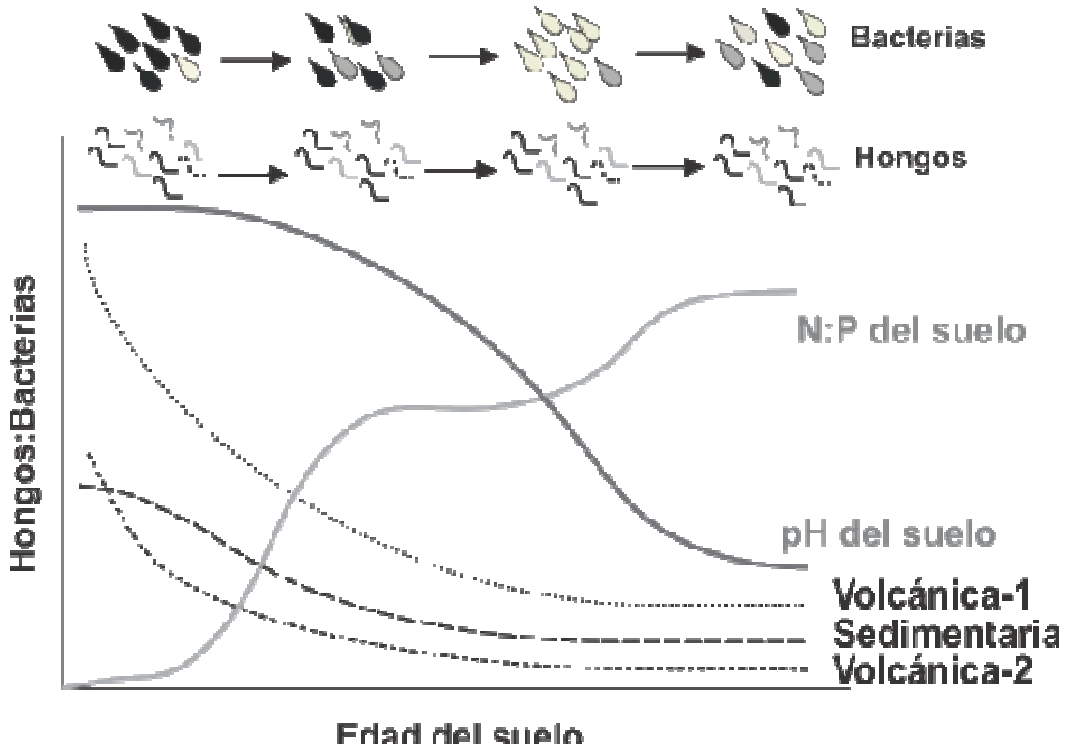


Figura 4. Cambio en la dominancia microbiana expresada como la razón Hongo:Bacteria en respuesta a los cambios en la razón N:P y el pH durante el desarrollo de los suelos en los tres tipos de cronosecuencias.